PATENT COOPERATION TREATY

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 May 2000 (24.05.00)	
International application No. PCT/US99/20948	**Applicant's or agent's file reference** 00530/089W01
International filing date (day/month/year) 14 September 1999 (14.09.99)	**Priority date** (day/month/year) 14 September 1998 (14.09.98)
Applicant DUKE-COHAN, Jonathan, S. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

06 April 2000 (06.04.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was [ ] was not made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer S. Mafla Telephone No.: (41-22) 338.83.38

1 - [illegible]

PATENT COOPERATION TREATY

REC'D 07 FEB 2001
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

4

Applicant's or agent's file reference 00530/089WO1	**FOR FURTHER ACTION** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/20948	International filing date *(day/month/year)* 14 SEPTEMBER 1999	Priority date *(day/month/year)* 14 SEPTEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant DANA-FARBER CANCER INSTITUTE		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 06 APRIL 2000	Date of completion of this report 05 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence For GERALD R. EWOLDT Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998) ★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/20948

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:

pages 1-52, as originally filed

pages NONE, filed with the demand

pages NONE, filed with the letter of

[X] the claims:

pages 53-59, as originally filed

pages NONE, as amended (together with any statement) under Article 19

pages NONE, filed with the demand

pages NONE, filed with the letter of

[X] the drawings:

pages 1-22, as originally filed

pages NONE, filed with the demand

pages NONE, filed with the letter of

[X] the sequence listing part of the description:

pages NONE, as originally filed

pages NONE, filed with the demand

pages NONE, filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language ______ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20948

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 4-5, 7-19, 28-37

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 4-5, 7-19, 28-37 .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	none	YES
	Claims	1-3, 6, 20-27	NO
Inventive Step (IS)	Claims	none	YES
	Claims	1-3, 6, 20-27	NO
Industrial Applicability (IA)	Claims	1-3, 6, 20-27	YES
	Claims	none	NO

2. citations and explanations (Rule 70.7)

Claims 1-3, 6, and 20-27 lack novelty under PCT Article 33(2) as being anticipated by Duke-Cohan et al. (1995) or Duke-Cohan et al (1996).

The Duke-Cohan et al. references describe the isolation and characterization of both the membrane and soluble forms of the polypeptides encoded by the claimed DNA nucleic acid sequences. Further characterization, i.e., sequencing, adds no novelty to the invention. The amino acid or nucleotide sequence of a polypeptide, or the DNA encoding said polypeptide respectively, is an inherent property of said polypeptide or DNA.

Absent evidence to the contrary, the attractions of the claimed invention are considered to be soluble and membrane Novel Form of Dipeptidylpeptidase IV, as taught by Duke-Cohan et al. (1995), or DPPT-L, as taught in the Duke-Cohan et al. (1996), described supra.

Claims 1-3, 6, and 20-27 meet the criteria set out in PCT Article 33(4) for industrial applicability. The claimed nucleic acids would possess utility in being used to express recombinant polypeptides for use in a method for treatment of a subject in need of an enhanced immune response.

-------------------- NEW CITATIONS --------------------

none

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20948

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C07H 21/04; C12N 15/63, 1/21, 15/00 and US Cl.: 536/23.5, 23.4; 435/320.1, 252.1, 69.7

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
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BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
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CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: FRASER, JANIS K.
FISH AND RICHARDSON P.C.
225 FRANKLIN STREET
BOSTON, MASSACHUSETTS 02110-2804
UNITED STATES OF AMERICA

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing *(day/month/year)*	07 FEB 2000
Applicant's or agent's file reference 00530/089WO1	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US99/20948	International filing date *(day/month/year)* 14 SEPTEMBER 1999
Applicant DANA-FARBER CANCER INSTITUTE	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer D. Lawrence Fa GERALD R. EWOLDT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/220 (January 1994)★ *(See notes on accompanying sh*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 00530/089WO1	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
Inter: .onal application No. PCT/US99/20948	International filing date *(day/month/year)* 14 SEPTEMBER 1999	(E: .st) Priority Date *(day/month/year)* 14 SEPTEMBER 1998
Applicant DANA-FARBER CANCER INSTITUTE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [X] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been c.. ..lished by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. ______
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20948

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6, 20-27

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20948

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; C12N 15/63, 1/21, 15/00
US CL :536/23.5, 23.4; 435/320.1, 252.1, 69.7
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.4; 435/320.1, 252.1, 69.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, medline, embase, BIOSIS, caplus,
kEYWORDS: attractin, DPPT-L

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DUKE-COHAN et al. A Novel Form of Dipeptidylpeptidase IV Found in Human Serum. J. Biol. Chem. June 1995, Vol. 270, No. 23, pages 14107-14114, see entire document.	1-3, 6, 20-27
Y	DUKE-COHAN et al. Serum High Molecular Weight Dipeptidyl Peptidase IV (CD26) Is Similar To A Novel Antigen DPPT-L Released From Activated T Cells. J. Immunol. 1996, Vol. 156, pages 1714-1721, see entire document.	1-3, 6, 20-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
06 JANUARY 2000

Date of mailing of the international search report
07 FEB 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
D. Lawrence for
GERALD R. EWOLDT
Telephone No. (703) 308-0196

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07H 21/04, C12N 15/63, 1/21, 15/00

A1

(11) International Publication Number: WO 00/15651

(43) International Publication Date: 23 March 2000 (23.03.00)

(21) International Application Number: PCT/US99/20948

(22) International Filing Date: 14 September 1999 (14.09.99)

(30) Priority Data:
60/100,137 14 September 1998 (14.09.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/100,137 (CON)
Filed on 14 September 1998 (14.09.98)

(71) Applicant (*for all designated States except US*): DANA-FARBER CANCER INSTITUTE [US/US]; 44 Binney Street, Boston, MA 02115 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): DUKE-COHAN, Jonathan, S. [IL/US]; 93 Parker Avenue, Newton Highlands, MA 02161 (US). SCHLOSSMAN, Stuart, F. [US/US]; 1 Fox Place, Newton Centre, MA 02195 (US).

(74) Agent: FRASER, Janis, K.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: REGULATION OF IMMUNE RESPONSES BY ATTRACTIN

(57) Abstract

The invention features attractin polypeptides and nucleic acids encoding them. The attractin polypeptides are useful for enhancing immune responses.

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REGULATION OF IMMUNE RESPONSES BY ATTRACTIN

Background of the Invention

The present invention relates to agents that regulate immune responses.

Analysis of *in vitro* immune responses allows basic interactions between cells and soluble modulators to be studied, but interpretations may be difficult to extend to actual responses *in vivo*, where reactions occur in complex cellular environments with constant dynamic modification of the extracellular environment. An important role is played by the extracellular matrix which interacts with adhesion structures on the surface of immune cells, directing cell migration, localization and clustering and subsequently influences the activity of local cytokines and lymphokines [Shimizu et al. (1991) *FASEB J.* 5, 2292-2299; Gilat et al. (1996) *Immunol. Today* 17, 16-20]. The passage of activated leukocytes between endothelial cells and their migration through the extracellular matrix to sites of inflammation is facilitated by the upregulated surface expression of several adhesion molecules and proteases [Hauzenberger et al. (1995) *Crit. Rev. Immunol.* 15, 285-316].

On activated T cells, one of the most prominently expressed proteases is CD26, which is a marker of T lymphocytes capable of migrating across endothelial barriers [Masuyama et al. (1992) *J. Immunol.* 148, 1367-1374; Brezinschek et al. (1995) *J. Immunol.* 154, 3062-3077] and has a collagen-binding domain [Loster et al. (1995) *Biochem. Biophys. Res. Commun.* 217, 341-348]. CD26 is now known to be identical to both dipeptidyl peptidase IV (DPPIV) and adenosine deaminase binding protein [Kameoka et al. (1993) *Science* 261, 466-469]. The understanding of the multifunctionality of CD26, which is the prototype for a family of related molecules

which includes Fibroblast Activation Protein [Scanlan et al. (1994) *Proc. Natl. Acad. Sci.* USA 91, 5657-5661], DPPIV [Wada et al. (1992) *Proc. Natl, Acad. Sci. USA* 89, 197-201] and Seprase [Goldstein et al. (1997) *Biochim. Biophys. Acta* 1361, 11-19; Pineiro-Sanchez et al. (1997) *J. Biol. Chem.* 272, 7595-7601], has expanded to include T lymphocyte costimulatory activity, where it enhances immune responses channeled through the CD3/T cell receptor complex [Dang et al. (1990) J. Immunol. 144, 4092-4100].

A soluble serum form of DPPIV had previously been identified [Tanaka et al. (1994) *Proc. Natl. Acad. Sci.* USA 91, 3082-3086], and its circulating levels were determined to be related to the ability of peripheral blood mononuclear cells (PBMC) to react *in vitro* to recall antigens such as tetanus toxoid. Based on this activity, it was conjectured that the identified soluble serum protein was a soluble form of CD26. However, upon purification of the protein, its glycosylated form was found to have a molecular weight of 175 kDa, and therefore, it was distinct from the 105 kDa glycosylated form of DPPIV/CD26 [Duke-Cohan et al. (1995) *J. Biol. Chem.* 270, 14107-14114]. The soluble serum protein having DPPIV activity was designated DPPT-L. DPPT-L appeared to be related to CD26 in that it displayed some CD26 antigenic epitopes, it was rapidly expressed as a T lymphocyte activation antigen, after 48-72 hr it was released from the lymphocyte membrane, and it could upregulate recall antigen-specific T cell responses in a manner similar to that of CD26 [Duke-Cohan et al. (1996) *J. Immunol.* 156, 1714-1721].

Summary of the Invention

The invention features four isolated forms of the human attractin polypeptide. These are soluble

attractin-1 (previously named DPPT-L in the mistaken belief that it was related to, and even a soluble form of, DPPIV/CD26), membrane attractin-1, soluble attractin-2, and membrane attractin-2. Text that refers to attractin without specifying soluble versus membrane or attractin-1 versus attractin-2 is pertinent to all forms of attractin. Membrane attractin differs from soluble attractin in that it has a transmembrane domain and a cytoplasmic domain. Attractin-2 differs from attractin-1 in that it contains a 74-amino acid insert in the N-terminal part of the polypeptide. The attractin molecules serve to enhance immune response by promoting macrophage and monocyte spreading in the presence of T cells. The invention also includes nucleic acid molecules encoding attractin polypeptides, vectors containing the nucleic acid molecules, and cells transformed with the vectors. In addition, the invention includes methods of enhancing or inhibiting immune responses and methods of identifying compounds that enhance or inhibit immune responses.

Specifically, the invention features an isolated DNA including: (a) a nucleic acid sequence that encodes a polypeptide that enhances spreading of a macrophage or a monocyte and that hybridizes under stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18; or (b) a complement of this nucleic acid sequence. The nucleic acid sequence included in the isolated DNA will be at least 10 bp, 15 bp, 25 bp, 50 bp, 75 bp, 100 bp, 125 bp, 150 bp, 175 bp, 200 bp, 250 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 1,500 bp, 2,000 bp, 3,000 bp, or 4,000 bp long. The nucleic acid sequence can encode a polypeptide that includes the amino sequence

of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:18. Examples include nucleotide sequences SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, and SEQ ID NO:19.

An isolated polypeptide within the invention can include the amino acid sequence of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:18, or can differ from one of these sequences solely by one or more conservative amino acid substitutions. The polypeptides of the invention also embrace fusion proteins containing both (a) an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18, but lacking methionine at position 1 of said amino acid sequence; and (b) a heterologous leader peptide. Also included are isolated nucleic acid molecules encoding the fusion proteins.

The invention features methods of enhancing spreading of a macrophage or a monocyte *in vitro*. These methods include coculturing a monocyte or a macrophage and a T cell with one or more of the following agents: (a) an isolated attractin polypeptide with the amino acid sequence of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:18; (b) a functional fragment of one or more of these attractin polypeptides; or (c) the polypeptide or the functional fragment, but with at least one conservative amino acid substitution.

The above polypeptides and nucleic acids can be used in a method of treating a mammal (e.g., a human) in need of an enhanced immune response. The method includes the step of delivering, to a tissue of a mammal where the tissue contains T cells and macrophages or monocytes, one of the above agents. The method can involve administration of the agent or a nucleic acid encoding the agent to the mammal. The human can be one suspected of being immunodeficient (e.g., one having common variable immunodeficiency) and/or of having cancer; and

can be performed before, during, or after chemotherapy or radiation therapy.

The invention also embodies a method of inhibiting spreading of a macrophage or a monocyte in a mammal. The method includes administering to the mammal an isolated compound that binds to an attractin polypeptide, and interferes with its function. The product can be an antibody and the mammal can be a human, e.g., a human suspected of having an autoimmune disease or a transplant recipient.

The invention also features vectors including any of the isolated DNAs of the invention, e.g. a vector in which the nucleic acid sequence encoding the relevant polypeptide is operably linked to a regulatory element which allows expression of the coding sequence in a cell. Cultured cells including the above vectors can be used in methods of producing any of the polypeptides of the invention. These methods include culturing the appropriate cell and purifying the polypeptide from it.

The invention also features a method of identifying a compound that inhibits an immune response. The method includes: a) providing an isolated polypeptide containing an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18, or the same amino acid sequence but with one or more conservative amino acid substitutions; b) co-culturing a T cell and a macrophage or a monocyte with the isolated polypeptide and the test compound; c) determining whether the test compound inhibits spreading of the macrophage or the monocyte, as an indication that the test compound inhibits an immune response. Alternatively, the method can include: a) providing a test compound; b) combining the test compound, a T cell, a macrophage or a monocyte, and the isolated polypeptide; and c) determining whether the test compound enhances

spreading of the macrophage or the monocyte, as an indication that the test compound inhibits an immune response.

Also within the invention is a method of identifying a compound that enhances an immune response. The method includes: a) providing a test compound; b) combining the test compound, a T cell, a macrophage or a monocyte, and an isolated polypeptide of the invention; and c) determining whether the test compound enhances spreading of the macrophage or the monocyte, as an indication that the test compound inhibits an immune response. Alternatively, the method can include: a) providing the isolated polypeptide; b) co-culturing a T cell and a macrophage or a monocyte with the isolated polypeptide and the test compound; c) determining whether the test compound inhibits spreading of the macrophage or the monocyte, as an indication that the test compound inhibits an immune response.

Also within the invention is an antibody (e.g., a scFv) that binds to a polypeptide with the amino acid sequence of SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:18, but does not bind to CD26 or to a polypeptide with the sequence of SEQ ID NO:2.

The invention also features an *ex vivo* method of treating a mammal (e.g., a human patient) in need of an enhanced immune response. The method includes: a) providing a recombinant cell which is the progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding an "agent" or a functional fragment of the agent so that the cell expresses the agent or functional fragment; and b) administering the cell to the mammal. The "agent" is: (i) an attractin polypeptide that includes the amino acid sequence of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:18; (ii) a functional fragment of the attractin

polypeptide; or (iii) the polypeptide or the functional fragment, but with one or more conservative amino acid substitutions.

Another aspect of the invention is an isolated functional attractin fragment including at least amino acid residues 31-104 of SEQ ID NO:12 or SEQ ID NO:18, amino acid residues 1279-1301 of SEQ ID NO:12, amino acid residues 1219-1429 of SEQ ID NO:12, or amino acid residues 1302-1429 of SEQ ID NO:12.

"Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. The invention also features attractin polypeptides with conservative substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, alanine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

The term "isolated" polypeptide or peptide fragment as used herein refers to a polypeptide or a peptide fragment which either has no naturally-occurring counterpart (e.g., a peptidomimetic), or has been substantially separated or purified from components which naturally accompany it, e.g., in tissues such as pancreas, liver, spleen, ovary, testis, muscle, joint tissue, neural tissue, gastrointestinal tissue, or body fluids such as blood, serum, or urine. Typically, the polypeptide or peptide fragment is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide (or peptide fragment thereof) of the invention is at least 80%, more

preferably at least 90%, and most preferably at least 99%, by dry weight, the polypeptide (or the peptide fragment thereof), respectively, of the invention. Thus, for example, a preparation of polypeptide x is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, polypeptide x. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, a synthetic polypeptide is by definition "isolated."

An isolated polypeptide (or peptide fragment) of the invention can be obtained, for example, by extraction from a natural source (e.g., from human tissues or bodily fluids); by expression of a recombinant nucleic acid encoding the peptide; or by chemical synthesis. A peptide that is produced in a cellular system different from the source from which it naturally originates is "isolated," because it will be separated from components which naturally accompany it. The extent of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

An "isolated DNA" means a DNA which either (a) has a non-naturally occurring sequence (e.g., a cDNA from a gene that naturally has introns), or (b) has a naturally occurring (i.e., genomic) sequence, but is free of the genes that flank the sequence in the genome of the organism in which the gene of interest naturally occurs. The term "isolated DNA" therefore includes a recombinant DNA incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote at a site other than the site at which it occurs naturally. It also includes a separate molecule such as a cDNA; a genomic fragment; a fragment produced by polymerase chain reaction (PCR); a

restriction fragment; a DNA encoding a non-naturally occurring mutein, fusion protein, or fragment of a given protein; or a nucleic acid which is a degenerate variant of a naturally occurring nucleic acid. In addition, it includes a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein.

"Spreading" of a macrophage or a monocyte, which occurs after exposure of the macrophage or monocyte to an attractin protein and a T cell, involves flattening of the macrophage or monocyte on a surface, expansion of the macrophage's or monocyte's margins, and an increase in the cell's surface area. The spread macrophage or monocytes may produce cellular processes visible microscopically. Macrophages and monocytes that have spread can be distinguished from, e.g., fibroblasts or T cells, by their expression of surface CD14.

As used herein, a "fragment" of an attractin polypeptide contains part but not all of the full-length polypeptide. Generally, fragments will be five or more amino acids in length. An antigenic fragment has the ability to be recognized and bound by an antibody.

As used herein, a "functional fragment" of an attractin polypeptide is a fragment of the polypeptide that has the ability to induce spreading of a macrophage or a monocyte in the presence of a T cell. Methods of establishing whether a fragment of an attractin molecule is functional are based upon those described herein for full-length polypeptides. For example, fragments of interest can be made by either recombinant, synthetic, or proteolytic digestive methods. Such fragments can then be isolated and tested for their ability to enhance spreading of macrophages or monocytes by procedures described herein.

As used herein, "operably linked" refers to an expression control sequence (e.g., a promoter, enhancer,

or the like) linked to a coding sequence in a manner that permits the expression control sequence to control expression of the coding sequence.

As used herein, the term "antibody" refers not only to whole antibody molecules, but also to antigen-binding fragments, e.g., Fab, $F(ab')_2$, Fv, and single chain Fv fragments. Also included are chimeric antibodies.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., enhancing immune responses in mammalian subjects, will be apparent from the following description, from the drawings and from the claims.

Brief Description of the Drawings

Figs. 1A-1F are photomicrographs showing monocyte spreading and T cell clustering after a 48 hr incubation of peripheral blood mononuclear cells (PBMC) with various concentrations of purified, natural soluble attractin-1. PBMC were incubated with no soluble attractin-1 (Fig. 1A) or soluble attractin-1 at a concentration of 0.5 μg/ml (Fig. 1B), 1μg/ml (Fig. 1C), 2 μg/ml (Fig. 1D), 5 μg/ml

(Fig. 1E), or 10 μg/ml (Fig. 1F). Figs. 1G-1I are photomicrographs of cells from cultures to which soluble attractin-1 (10 μg/ml) had been added. The cells in the cultures, which were incubated for 48 hours, were PBMC separated into E^+ T lymphocytes (Fig. 1G), E^- monocytes/B cells (Fig. 1H), and E^+ T lymphocytes remixed with E^- monocytes/B cells (Fig. 1I).

Fig. 2 is a depiction of the amino acid sequence of soluble attractin-1 (SEQ ID NO:2). The sequences identified by N-terminal sequencing of tryptic and chymotryptic peptides are underlined.

Figs. 3A-3B are photographs showing attractin mRNA expression in Northern blots of multiple tissue mRNA (Fig. 3A) and resting and PHA-activated PBMC total RNA (Fig. 3B). Fig. 3C is a photograph showing an ethidium bromide stained electrophoretic gel of 3,164 bp attractin DNA fragments obtained by PCR using three independent cDNA libraries as sources of templates.

Figs. 4A-4C are diagrams showing the organization of soluble attractin-1 cDNA and peptide domains. Fig. 4A is a diagram of soluble attractin-1 cDNA. The bases shown in upper case at the origin represent bases satisfying the Kozak consensus. Fig. 4B shows a comparison of soluble attractin-1 protein domains and motifs with those of *C. elegans* F33C8.1 protein. The horizontal bars depict the position of cysteines shared by both sequences. Fig. 4C shows a comparison of the putative catalytic serine motif of soluble attractin-1 with the catalytic serine motifs of other serine proteases. The shaded box indicates agreement with the consensus, '#' or exclusion from the shaded boxes indicates conflict, and 'X' indicates satisfaction by any amino acid. The parentheses enclose amino acids any of which would satisfy the consensus.

Figs. 5A-5D are photomicrographs showing the intracellular localization of glycosylated soluble attractin-1 by immunogold electron microscopy using rabbit polyclonal antibody specific for soluble attractin-1; in resting T lymphocyte with no evidence of soluble attractin-1 expression (Fig. 5A); in T cells activated for 48 hr with PHA and in which, soluble attractin-1 is expressed in large vacuoles (Fig. 5B); in vesicles in which soluble attractin-1 localizes in an electron dense core (Fig. 5C); and in vesicles containing soluble attractin-1 breaking open at the cell surface, releasing soluble attractin-1 (Fig. 5D).

Figs. 6A-6C are photographs showing expression of recombinant soluble attractin-1 and immunoprecipitation by antibody specific for natural soluble attractin-1. Fig. 6A shows a photograph of an SDS-PAGE gel of soluble attractin-1 transcribed and translated *in vitro* in the absence or presence of glycosyl transferases. Fig. 6B is a photograph of a Western blot (developed with antibody specific for myc) of lysates of 293T cells transiently-transfected with pSecTag2B-soluble attractin-1 or pSecTag2B vector control. Fig. 6C shows a photograph of an SDS-PAGE gel of soluble attractin-1 precipitated with pre-immune serum or polyclonal antibody specific for soluble attractin from lysates of CHO cells stably transfected with pSecTag2B-attractin.

Figs. 7A-7D are photomicrographs showing that recombinant soluble attractin-1 mediates monocyte/macrophage spreading and T cell clustering. Resting PBMC were incubated for 48 hr without soluble attractin-1 (Fig. 7A) or with soluble attractin-1 at a concentration of 1 μg/ml (Fig. 7B), 2 μg/ml (Fig. 7C), or 5 μg/ml (Fig. 7D).

Fig. 8 is a depiction of the nucleotide sequence of soluble attractin-1 cDNA (SEQ ID NO:1).

Fig. 9 is a depiction of the amino acid sequence of membrane attractin-1 (SEQ ID NO:10).

Fig. 10 is a depiction of the nucleotide sequence of membrane attractin-1 encoding cDNA (SEQ ID NO:11).

Fig. 11 is a depiction of the amino acid sequence of soluble attractin-2 (SEQ ID NO:18).

Fig. 12 is a depiction of the nucleotide sequence of soluble attractin-2 encoding cDNA (SEQ ID NO:19).

Fig. 13 is a depiction of the amino acid sequence of membrane attractin-2 (SEQ ID NO:12).

Fig. 14 is a depiction of the nucleotide sequence of membrane attractin-2 encoding cDNA (SEQ ID NO:13).

Fig. 15 is a series of fluorescence flow cytometric histograms showing the expression of MHC class I, MHC class II, B7.1, B7.2, CD11a, CD29, CD54, and CD58 molecules on the surface of total peripheral blood leukocytes ("All"), lymphocytes ("Lymph"), and monocytes ("Mono"), prior to culture ("DO"), and after a 72 hr incubation of peripheral blood leukocytes with either recombinant soluble attractin-1 ("X") or granulocyte macrophage colony-stimulating factor ("GM-CSF").

Description of the Invention

The invention is based, in part, on the cloning of cDNA molecules encoding different overlapping regions of human soluble attractin-1, membrane attractin-1, soluble attractin-2, and membrane attractin-2. Contrary to initial indications when soluble attractin-1 was first studied, It was determined that there is no significant amino acid sequence homology between attractin and CD26, or any other characterized human protein. Both purified serum-derived and recombinant soluble attractin-1 induce the spreading of macrophages and monocytes that become the focus for the clustering of non-proliferating T lymphocytes. T lymphocytes use soluble attractin-1, at least, to marshall together the cells required to form a

cluster of co-operating immune cells. Since membrane attractin-1 and the putative attractin-2 molecules contain all the functional domains of soluble attractin, it is likely that they have similar activity. Thus, attractin has an important role in the regulation of the immune response.

The various forms of attractin are encoded by alternatively spliced mRNA molecules transcribed from a single gene. The 134-kDa soluble attractin-1 protein includes a putative serine protease catalytic serine at amino acid residue 26, four EGF-like motifs, a CUB domain, a C-type lectin domain and a domain homologous with the ligand-binding region of the common cytokine receptor γ chain (Fig. 4C). Except for the latter two domains, the overall structure shares high homology with the *C. elegans* F33C8.1 protein, suggesting that attractin has evolved new domains and functions in parallel with the development of cell-mediated immunity. Membrane attractin-1 contains all these domains and, in addition, C-terminal transmembrane and cytoplasmic domains. Furthermore, attractin-2 has a 74 amino acid insertion, immediately after amino acid residue 30 of attractin-1. This insertion is likely to be important as a glycosylation targeting motif (e.g., a Golgi-targeting motif.

The experiments described in Examples 2, 5, and 10 below show that soluble attractin-1 mediates an interaction between T lymphocytes and monocytes, leading to adherence and spreading of the monocytes which become a focus for T lymphocyte clustering. No difference was observed in attractin mRNA expression between resting and activated PBMC, indicating that a regulatory step exists between transcription and glycosylation rather than in induction of *de novo* mRNA synthesis. Despite extensive N-glycosylation of the isolated serum (soluble)

attractin, there are no consensus signal sequences encoded in the cDNA sequences encoding attractin. Another protein may chaperone attractin through the Golgi complex and endoplasmic reticulum. However, several proteins are known to be secreted without a signal peptide, including FGF-9 [Miyamoto et al. (1993) *Mol. Cell. Biol*.13, 4251-4259], IL1-α and β [Rubartelli et al. (1990) *EMBO J*. 9, 1503-1510], FGF-1 [Tarantini et al. (1995) *J. Biol. Chem*. 270, 29039-29042], FGF-2 [Mignatti et al. (1992) *J. Cell. Physiol*. 151, 81-93], and platelet-derived endothelial cell growth factor [Mignatti et al. (1992) *J. Cell. Physiol*. 151, 81-93]. This has led to the proposal of alternative secretory pathways with slow exocytic release from large cytoplasmic pools [Rubartelli et al. (1997) In *Unusual Secretory Pathways: from Bacteria to Man*, eds. Kuchler et al. (R.G. Landes Co., Austin, TX), pp. 87-114]. The electron microscopy results described above confirm that the early activated T lymphocyte secretion of soluble attractin, at least, results from vesicular release at the plasma membrane.

Expression of attractin on the surface of activated T cells could involve any of the forms of the protein described herein. Thus, for example, surface could be the membrane form of attractin (-1 or -2) bound via its transmembrane domain to the T cell membrane. Alternatively, it could be soluble attractin (-1 or -2) that has been secreted into the milieu of the T cell and then binds via a cell-surface receptor, or via a non-specific hydrophobic interaction, to the T cell. In addition, attractin on the T cell could be soluble attractin (-1 or -2) that is in transit from the cytoplasm to the exterior of the T cell. The invention is not limited by any particular mechanism of T cell surface expression of attractin.

Proteins, such as attractin, containing EGF-like motifs are usually involved in extracellular signalling or cell guidance [Davis, C.G. (1990) *New Biol.* 2, 410-419]. Attractin also contains a motif representing the ligand-binding region of the cytokine receptor common γ chain [D'Andrea et al. (1990) *Curr. Opin. Cell Biol.* 2, 648-651]. In overall structure and organization of domains, attractin most closely resembles the CUB-containing protein BMP1 (bone morphogenic protein-1) which influences cell interactions during development [Li et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 5127-5130]. The acronym "CUB" derives from the names of the three prototypic proteins (complement component-1 r/s (C1r/s), U-EGF (epidermal growth factor), and BMP-1). The C-type lectin domain recognizes carbohydrate and is characteristic of the selectin family of proteins involved in adhesion of leukocytes to vascular endothelia. This domain is also characteristic of proteins involved in endocytosis for antigen processing in macrophages and dendritic cells [Weis et al. (1996) *Ann. Rev. Biochem.* 65, 441-473].

There is a high level of identity between attractin and the 143 kDa *C. elegans* F33C8.1 protein. The potential γ-chain ligand binding motif and C-type lectin domain present in attractin are missing in the *C. elegans* transcript, suggesting an evolutionary development in which the human form incorporated these new domains in parallel with the development of cell-mediated immunity.

Like CD26, soluble attractin-1 alone is unable to induce cell proliferation, but is able to enhance the proliferative response of PBL to recall antigens such as tetanus toxoid [Duke-Cohan et al. (1995) *J. Biol. Chem.* 270, 14107-14114; Duke-Cohan et al. (1996) *J. Immunol.* 156, 1714-1721]. Therefore, it appears that soluble

attractin-1, and indeed the other attractin molecules described herein, modulate the interaction between T cells and macrophages and monocytes, permitting more rapid and/or more effective antigen presentation. It is likely that the minimal immunoregulatory unit consists of an antigen-presenting cell which acts as a focus for a cluster of T helper cells and effector cells [Stuhler et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 622-627]. The association of the three cell types is neither simultaneous nor random. Rather, the antigen-presenting cell clusters first with the helper T cells, and this cluster acts as a focus for recognition by effector cells [Ridge et al. (1998) *Nature* 393, 474-478]. In the absence of antigen, no proliferation occurs in soluble attractin-1-induced clusters of monocytes and T cells, but if a recall antigen such as tetanus toxoid is present, the clustering of cells maximizes the potential response to the antigen. Attractin may regulate local cytokine activity, either by influencing binding and presentation or by proteolytic modification. Soluble attractin-1 has recently been shown to cleave an N-terminal dipeptide which converts full-length RANTES 1-68 (consisting of amino acid residues 1-68), a potent monocyte chemoattractant, to RANTES 3-68 (consisting of residues 3-68), an equally potent inhibitor of monocyte chemotaxis [Proost et al. (1998) *J. Biol. Chem.* 273, 7222-7227]. Soluble attractin-1 has also been found to bind to macrophages and monocytes. It is possible that it is via this binding that attractin, in any of its forms, may regulate the activity of macrophages and monocytes. It could, for example, provide one of two or more requisite signals necessary for the induction of spreading and subsequent enhanced T-cell clustering. Alternatively, it could complement binding of another molecule to a receptor on macrophages/monocytes.

Furthermore, it could form a bridge between T cells and macrophages/monocytes. Since membrane attractin (-1 and -2) has a cytoplasmic domain, it is likely that binding of a putative ligand to an extracellular region of membrane attractin results in signalling to the T cell. It should be understood, however, that the instant invention is not limited by a particular mechanism of action. The concatenation in attractin of domains related to regulation of cell interactions together with domains related to lymphokine/cytokine binding, the rapid upregulation of attractin cell surface expression by activated T cells, and the clear effect upon T cell-monocyte/macrophage association all suggest that attractin, either as a normal circulating serum protein or as a membrane bound protein, plays a significant role in the immune response *in vivo*.

Attractin Nucleic Acid Molecules

The attractin nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand). Segments of these molecules are also considered within the scope of the invention, and can be produced, for example, by the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription. Preferably, the nucleic acid molecules encode polypeptides that, regardless of length, are soluble under normal physiological conditions the membrane forms would not be soluble.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same

polypeptide (for example, the polypeptides with SEQ ID NOS:2, 10, 12, and 18). In addition, these nucleic acid molecules are not limited to coding sequences, e.g., they can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. Thus, the nucleic acids can be those of a human, non-human primate (e.g., monkey) mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, dog, or cat.

In addition, the isolated nucleic acid molecules of the invention encompass segments that are not found as such in the natural state. Thus, the invention encompasses recombinant nucleic acid molecules, (for example, isolated nucleic acid molecules encoding any of the forms of attractin described herein) incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

Certain nucleic acid molecules of the invention are antisense molecules or are transcribed into antisense molecules. These can be used, for example, to down-regulate translation of attractin mRNA within a cell.

Techniques associated with detection or regulation of genes are well known to skilled artisans and such techniques can be used to diagnose and/or treat disorders associated with aberrant attractin expression. Nucleic acid molecules of the invention are discussed further below in the context of their therapeutic utility.

An attractin family gene or protein can be identified based on its similarity to the relevant

attractin gene or protein, respectively. For example, the identification can be based on sequence identity. The invention features isolated nucleic acid molecules which are at least 50% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to: (a) a nucleic acid molecule that encodes the polypeptide of SEQ ID NO:2, 10, 12, or 18; (b) the nucleotide sequence of SEQ ID NO:1, 11, 13, or 19; or (c) a nucleic acid molecule which includes a segment of at least: (i) 30 (e.g., at least 50, 60, 100, 125, 150, 175, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 2000, 3000, or 3540) nucleotides of SEQ ID NO:1; (ii) 30 (e.g., at least 50, 60, 100, 125, 150, 175, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 2000, 3000, 4000, or 4050) nucleotides of SEQ ID NO:11; (iii) 30 (e.g., at least 50, 60, 100, 125, 150, 175, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 2000, 3000, 4000, or 4250) nucleotides of SEQ ID NO:13; or (iv) 30 (e.g., at least 50, 60, 100, 125, 150, 175, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 2000, 3000, or 3800) nucleotides of SEQ ID NO:19. The invention also features nucleic acid molecules which include a nucleotide sequence encoding a polypeptide that is at least 65% (e.g., at least 70%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:2, 10, 12, or 18.

The determination of percent identity between two sequences is accomplished using the mathematical algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 90, 5873-5877, 1993. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) *J. Mol. Biol.* 215, 403-410. BLAST nucleotide searches are performed with the BLASTN program, score = 100, wordlength = 12 to obtain

nucleotide sequences homologous to attractin-encoding nucleic acids. BLAST protein searches are performed with the BLASTP program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to attractin. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25, 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used (*See* http://www.ncbi.nlm.nih.gov).

Hybridization can also be used as a measure of homology between two nucleic acid sequences. An attractin-encoding nucleic acid sequence, or a portion thereof, can be used as hybridization probe according to standard hybridization techniques. The hybridization of an attractin probe to DNA from a test source (e.g., a mammalian cell) is an indication of the presence of attractin DNA in the test source. Hybridization conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. Moderate hybridization conditions are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by one or more washes in 1 X SSC, 0.1% SDS at 50-60°C. Highly stringent conditions are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C.

The invention also encompasses: (a) vectors that contain any of the foregoing attractin-related coding sequences and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing attractin-related coding sequences operatively associated with any transcriptional/translational regulatory elements (examples of which are given below)

necessary to direct expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding an attractin polypeptide, nucleic acid sequences that are unrelated to nucleic acid sequences encoding attractin, such as molecules encoding a reporter, marker, or a signal peptide, e.g., fused to attractin; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention.

Recombinant nucleic acid molecules can contain a sequence encoding a soluble attractin membrane attractin, or attractin having an heterologous signal sequence. The full length attractin polypeptide, a domain of attractin, or a fragment thereof may be fused to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of attractin or a form that includes an exogenous polypeptide which facilitates secretion.

The transcriptional/translational regulatory elements referred to above and which are further described below, include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

Similarly, the nucleic acid can form part of a

hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, $G418^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being an attractin polypeptide and the second portion being, for example, the reporter described above or an immunoglobulin constant region.

The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding attractin (contained within SEQ ID NOS:2, 10, 12, or 18); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus expression vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with

recombinant plasmid expression vectors (for example, Ti plasmid) containing attractin nucleotide sequences; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter). Also useful as host cells are primary or secondary cells obtained directly from a mammal, transfected with a plasmid vector or infected with a viral vector.

<u>Polypeptides and Polypeptide Fragments</u>

The polypeptides of the invention include soluble attractin-1 and -2, membrane attractin-1 and -2, and functional fragments of these polypeptides. The polypeptides embraced by the invention also include fusion proteins which contain either full-length attractin (any of the forms) or a functional fragment of it fused to unrelated amino acid sequence. The unrelated sequences can be additional functional domains or signal peptides. Signal peptides are described in greater detail and exemplified below.

The polypeptides can be purified from natural sources (e.g., blood, serum plasma, tissues or cells such as T cells or any cell that naturally produces attractin). Smaller peptides (less than 50 amino acids long) can also be conveniently synthesized by standard chemical means. In addition, both polypeptides and peptides can be produced by standard *in vitro* recombinant DNA techniques and *in vivo* recombination/genetic recombination (e.g., transgenesis), using the nucleotide sequences encoding the appropriate polypeptides or peptides. Methods well known to those skilled in the art

can be used to construct expression vectors containing relevant coding sequences and appropriate transcriptional/translational control signals. See, for example, the techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd Ed.) [Cold Spring Harbor Laboratory, N.Y., 1989], and Ausubel et al., Current Protocols in Molecular Biology, [Green Publishing Associates and Wiley Interscience, N.Y., 1989].

Polypeptides and fragments of the invention also include those described above, but modified for *in vivo* use by the addition, the amino- and/or carboxyl-terminal ends, of a blocking agent to facilitate survival of the relevant polypeptide *in vivo*. This can be useful in those situations in which the peptide termini tend to be degraded by proteases prior to cellular uptake. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. This can be done either chemically during the synthesis of the peptide or by recombinant DNA technology by methods familiar to artisans of average skill.

Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to administration.

Also of interest are peptidomimetic compounds that are designed based upon the amino acid sequences of the functional peptide fragments. Peptidomimetic compounds are synthetic compounds having a three-dimensional

conformation (i.e., a "peptide motif") that is substantially the same as the three-dimensional conformation of a selected peptide. The peptide motif provides the peptidomimetic compound with the ability to induce macrophage/monocyte spreading in a manner qualitatively identical to that of the attractin functional peptide fragment from which the peptidomimetic was derived. Peptidomimetic compounds can have additional characteristics that enhance their therapeutic utility, such as increased cell permeability and prolonged biological half-life.

The peptidomimetics typically have a backbone that is partially or completely non-peptide, but with side groups that are identical to the side groups of the amino acid residues that occur in the peptide on which the peptidomimetic is based. Several types of chemical bonds, e.g., ester, thioester, thioamide, retroamide, reduced carbonyl, dimethylene and ketomethylene bonds, are known in the art to be generally useful substitutes for peptide bonds in the construction of protease-resistant peptidomimetics.

Methods of Therapy

The methods of the invention involve combining a macrophage/monocyte, an attractin molecule of the invention, and a T cell, in order to induce spreading of macrophages/monocytes. The T cell can be a CD4+ T cell or a CD8+ T cell. The attractin molecule can be added to the solution containing the cells or it can be expressed on the surface of a T cell, e.g., the T cell that is added to the combined attractin and macrophages/monocytes. The methods can be performed *in vitro, in vivo*, or *ex vivo*. *In vitro* application of attractin can be useful, for example, in basic scientific studies of immune mechanisms or for production of

macrophages with increased ability to activate T cells for use in studies on macrophage/monocyte function. Furthermore, attractin could be added to *in vitro* assays (e.g., in T cell proliferation assays) designed to test for immunity to an antigen of interest in a subject from which the T cells were obtained. Addition of attractin to such assays would be expected to result in a more potent, and therefore more readily detectable, *in vitro* response. However, the methods of the invention will preferably be *in vivo* or *ex vivo* (see below).

The attractin proteins and variants thereof are generally useful as immune response-stimulating therapeutics, as described in International Application No. WO 96/38550 (published December 5, 1996), which is incorporated by reference herein in its entirety. For example, the polypeptides of the invention can be used for treatment of disease conditions characterized by immunosuppression: e.g., cancer, AIDS or AIDS-related complex, other virally or environmentally-induced conditions, and certain congenital immune deficiencies. The compounds may also be employed to increase immune function that has been impaired by the use of radiotherapy of immunosuppressive drugs such as certain chemotherapeutic agents, and therefore are particularly useful when given in conjunction with such drugs or radiotherapy. These methods of the invention can be applied to a wide range of species, e.g., humans, non-human primates, horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, hamsters, rats, and mice.

In Vivo Approaches

In one *in vivo* approach, the attractin polypeptide (or a functional fragment thereof) itself is administered to the subject. Generally, the compounds of the invention will be suspended in a pharmaceutically-

acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. They are preferably delivered directly to an appropriate lymphoid tissue (e.g. spleen, lymph node, or mucosal-associated lymphoid tissue (MALT)). The dosage required depends on the choice of the route of administration, the nature of the formulation, the nature of the patient's illness, the subject's size, weight, surface area, age, and sex, other drugs being administered, and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100.0 μg/kg. Wide variations in the needed dosage are to be expected in view of the variety of polypeptides and fragments available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by i.v. injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (e.g., 2- or 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-, 150-, or more fold). Encapsulation of the polypeptide in a suitable delivery vehicle (e.g., ????? polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Alternatively, a polynucleotide containing a nucleic acid sequence encoding the attractin polypeptide or functional fragment can be delivered to an appropriate cell of the animal. Expression of the coding sequence will preferably be directed to lymphoid tissue of the subject by, for example, delivery of the polynucleotide

to the lymphoid tissue. This can be achieved by, for example, the use of a polymeric, biodegradable microparticle or microcapsule delivery vehicle, sized to optimize phagocytosis by phagocytic cells such as macrophages. For example, PLGA (poly-lacto-co-glycolide) microparticles approximately 1-10 μm in diameter can be used. The polynucleotide is encapsulated in these microparticles, which are taken up by macrophages and gradually biodegraded within the cell, thereby releasing the polynucleotide. Once released, the DNA is expressed within the cell. A second type of microparticle is intended not to be taken up directly by cells, but rather to serve primarily as a slow-release reservoir of nucleic acid that is taken up by cells only upon release from the micro-particle through biodegradation. These polymeric particles should therefore be large enough to preclude phagocytosis (i.e., larger than 5μm and preferably larger than 20μm). Microparticles useful for nucleic acid delivery, methods for making them, and methods of use are described in greater detail in U.S. Patent No. 5,783,567, incorporated herein by reference in its entirety.

Another way to achieve uptake of the nucleic acid is using liposomes, prepared by standard methods. The vectors can be incorporated alone into these delivery vehicles or co-incorporated with tissue-specific antibodies. Alternatively, one can prepare a molecular conjugate composed of a plasmid or other vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells [Cristiano et al. (1995), *J. Mol. Med.* 73, 479]. Alternatively, lymphoid tissue specific targeting can be achieved by the use of lymphoid tissue-specific transcriptional regulatory elements (TRE) such as a B lymphocyte, T lymphocyte, or dendritic cell specific TRE. Lymphoid tissue specific TRE are known

[Thompson et al. (1992), *Mol. Cell. Biol*. 12, 1043-1053; Todd et al. (1993), *J. Exp. Med*. 177, 1663-1674; Penix et al. (1993), *J. Exp. Med*. 178, 1483-1496]. Delivery of "naked DNA" (i.e., without a delivery vehicle) to an intramuscular, intradermal, or subcutaneous site, is another means to achieve *in vivo* expression.

In the relevant polynucleotides (e.g., expression vectors) the nucleic acid sequence encoding the attractin polypeptide or functional fragment of interest with an initiator methionine and optionally a targeting sequence is operatively linked to a promoter or enhancer-promoter combination.

Short amino acid sequences can act as signals to direct proteins to specific intracellular compartments. For example, hydrophobic signal peptides (e.g., MAISGVPVLGFFIIAVLMSAQESWA (SEQ ID NO:14)) are found at the amino terminus of proteins destined for the ER. While the sequence KFERQ (SEQ ID NO:15) (and other closely related sequences) is known to target intracellular polypeptides to lysosomes, other sequences (e.g., MDDQRDLISNNEQLP (SEQ ID NO:16) direct polypeptides to endosomes. In addition, the peptide sequence KDEL (SEQ ID NO:17) has been shown to act as a retention signal for the ER. Each of these signal peptides, or a combination thereof, can be used to traffic the attractin polypeptides or functional fragments of the invention as desired. DNAs encoding the attractin polypeptides or functional fragments containing targeting signals will be generated by PCR or other standard genetic engineering or synthetic techniques. Targeting sequences are described in greater detail in U.S. Patent No. 5,827,516, incorporated herein by reference in its entirety.

A promoter is a TRE composed of a region of a DNA molecule, typically within 100 basepairs upstream of the point at which transcription starts. Enhancers provide

expression specificity in terms of time, location, and level. Unlike a promoter, an enhancer can function when located at variable distances from the transcription site, provided a promoter is present. An enhancer can also be located downstream of the transcription initiation site. To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the peptide or polypeptide between one and about fifty nucleotides downstream (3') of the promoter. The coding sequence of the expression vector is operatively linked to a transcription terminating region.

Suitable expression vectors include plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses and adeno-associated viruses, among others.

Polynucleotides can be administered in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to a human, e.g., physiological saline. A therapeutically effective amount is an amount of the polynucleotide which is capable of producing a medically desirable result in a treated animal. As is well known in the medical arts, the dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for administration of polynucleotide is from approximately 10^6 to 10^{12} copies of the polynucleotide molecule. This dose can be repeatedly administered, as needed. Routes of administration can be any of those listed above.

Ex Vivo Approaches

Peripheral blood leukocytes can be withdrawn from the patient or a suitable donor and treated *ex vivo* with the attractin protein or polypeptide fragment (whether in soluble form or attached to a sold support by standard methodologies). The leukocytes containing newly-activated monocytes are then introduced into the same or a different patient.

An alternative *ex vivo* strategy can involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding an attractin polypeptide or functional fragment-encoding nucleic acid sequences described above. The transfected or transduced cells are then returned to the subject. While such cells would preferably be lymphoid cells, they could also be any of a wide range of types including, without limitation, fibroblasts, bone marrow cells, macrophages, monocytes, dendritic cells, epithelial cells, endothelial cells, keratinocytes, or muscle cells in which they act as a source of the attractin polypeptide or functional fragment for as long as they survive in the subject. The use of lymphoid cells would be particular advantageous in that such cells would be expected to home to lymphoid tissue (e.g., lymph nodes or spleen) and thus the attractin polypeptide or functional fragment would be produced in high concentration at the site where they exert their effect, i.e., enhancement of an immune response. In addition, if T cells are used, the T cell expressing the exogenous attractin molecule can be the T cell that is required, together with attractin, to induce spreading and activation of macrophages or monocytes. The attractin can be secreted by the T cell or expressed on the surface of the T cell. The same genetic constructs and trafficking sequences described

for the *in vivo* approach can be used for this *ex vivo* strategy.

The *ex vivo* methods include the steps of harvesting cells from a subject, culturing the cells, transducing them with an expression vector, and maintaining the cells under conditions suitable for expression of the attractin polypeptide or functional fragment. These methods are known in the art of molecular biology. The transduction step is accomplished by any standard means used for *ex vivo* gene therapy, including calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced are then selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells may then be lethally irradiated (if desired) and injected or implanted into the patient.

Methods of Screening for Compounds that Inhibit or Enhance Immune Responses.

The invention provides methods for testing compounds (small molecules or macromolecules) that inhibit or enhance an immune response. Such a method could involve, e.g., culturing macrophages or monocytes with: (a) any of the attractin molecules of the invention, (b) T cells; and (c) a candidate compound. The attractin molecule can be in solution or membrane bound (e.g., expressed on the surface of the T cells) and it can be natural or recombinant. Furthermore, it can be a functional fragment of an attractin molecule. Compounds that inhibit macrophage or monocyte spreading will likely be compounds that inhibit an immune response

while those that enhance macrophage and monocyte spreading will likely be compounds that enhance an immune response. Instead of testing for an effect of a compound on macrophage or monocyte spreading, the ability of the compound to inhibit or enhance induction of B7.1 or MHC class II molecule expression on the macrophage could also be measured.

The invention also relates to using attractin or functional fragments thereof to screen for immunomodulatory compounds that can interact with attractin. One of skill in the art would know how to use standard molecular modeling or other techniques to identify small molecules that would bind to the unique sites of attractin described herein. On such example is provided in Broughton (1997) Curr. Opin. Chem. Biol. 1, 392-398.

A candidate compound whose presence requires at least 1.5-fold (e.g., 2-fold, 4-fold, 6-fold, 10-fold, 150-fold, 1000-fold, 10,000-fold, or 100,000-fold) more attractin in order to achieve macrophage or monocyte spreading than in the absence of the compound can be useful for inhibiting an immune response. On the other hand, a candidate compound whose presence requires at least 1.5 fold (e.g., 2-fold, 4-fold, 6-fold, 10-fold, 100-fold, 1000-fold, 10,000 fold, or 100,000-fold) less attractin to achieve macrophage or monocyte spreading than in the absence of the compound can be useful for enhancing an immune response. Compounds capable of interfering with or modulating attractin function are good candidates for immunosuppressive immunoregulatory agents, e.g., to modulate an autoimmune response or suppress allogeneic or xenogeneic graft rejection.

Attractin Antibodies

The invention features antibodies that bind to any or all of the described attractin polypeptides or fragments of such polypeptides. Such antibodies can be polyclonal antibodies present in the serum or plasma of animals (e.g., mice, rabbits, rats, guinea pigs, sheep, horses, goats, cows, or pigs) which have been immunized with the relevant attractin polypeptide or peptide fragment using methods, and optionally adjuvants, known in the art. Such polyclonal antibodies can be isolated from serum or plasma by methods known in the art. Monoclonal antibodies that bind to the above polypeptides or fragments are also embodied by the invention. Methods of making and screening monoclonal antibodies are well known in the art.

Once the desired antibody-producing hybridoma has been selected and cloned, the resultant antibody can be produced in a number of methods known in the art. For example, the hybridoma can be cultured *in vitro* in a suitable medium for a suitable length of time, followed by the recovery of the desired antibody from the supernatant. The length of time and medium are known or can be readily determined.

Additionally, recombinant antibodies specific for attractin, such as chimeric and humanized monoclonal antibodies comprising both human and non-human portions, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example, using methods described in Robinson et al., International Patent Publication PCT/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., PCT Application WO 86/01533; Cabilly et al., U.S. Patent No. 4,816,567;

Cabilly et al., European Patent Application 125,023; Better et al. (1988) *Science* 240, 1041-43; Liu et al. (1987) *J. Immunol.* 139, 3521-26; Sun et al. (1987) *PNAS* 84, 214-18; Nishimura et al. (1987) *Canc. Res.* 47, 999-1005; Wood et al. (1985) *Nature* 314, 446-49; Shaw et al. (1988) *J. Natl. Cancer Inst.* 80, 1553-59; Morrison, (1985) *Science* 229, 1202-07; Oi et al. (1986) *BioTechniques* 4, 214; Winter, U.S. Patent No. 5,225,539; Jones et al. (1986) *Nature* 321, 552-25; Veroeyan et al. (1988) *Science* 239, 1534; and Beidler et al. (1988) *J. Immunol.* 141, 4053-60.

Also included within the scope of the present invention are antibody fragments and derivatives which contain at least the functional portion of the antigen binding domain of an antibody that binds specifically to attractin. Antibody fragments that contain the binding domain of the molecule can be generated by known techniques. For example, such fragments include, but are not limited to: $F(ab')_2$ fragments which can be produced by pepsin digestion of antibody molecules; Fab fragments which can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments; and Fab fragments which can be generated by treating antibody molecules with papain and a reducing agent. See, e.g., National Institutes of Health, 1 Current Protocols In Immunology, Coligan *et al.*, ed. §§ 2.8, 2.10 (Wiley Interscience, 1991). Antibody fragments also include Fv (e.g., single chain Fv (scFv)) fragments, *i.e.*, antibody products in which there are no constant region amino acid residues. Such fragments can be produced, for example, as described in U.S. Patent No. 4,642,334.

The following examples are meant to illustrate, not limit, the invention.

Example 1. Materials and Methods

Cell techniques: PBMC, erythrocyte rosetting (E^+) T cells and erythrocyte non-rosetting (E^-) monocytes/B cells were purified as described previously [Morimoto et al. (1985) *J. Immunol.* 134, 3762-3769]. CHO ($dhfr^-$) cells and the Jurkat T cell line were obtained from the American Type Culture Collection (ATCC, Manassas, VA). 293T cells were obtained from Dr. B. Mayer (Children's Hospital, Boston, MA). All cell lines were maintained in RPMI 1640 containing 10% fetal bovine serum. For assessing the biological effects of soluble attractin-1, leukocytes were cultured in serum-free AIM V medium (Life Technologies Inc., Gaithersburg, MD) in 48-well plates (Costar, Cambridge, M). For cell activation, E^+ lymphocytes (10^6/ml) supplemented with 0.1% E^- cells were incubated in AIM V medium together with phytohemagglutinin (PHA, 1 μg/ml; Murex, Dartford, U.K.) for 48 hr. Cell proliferation was assessed using [^{3}H]-thymidine incorporation as described previously [Duke-Cohan et al. (1995) *J. Biol. Chem.* 270, 14107-14114].

RNA/DNA preparation and analysis: mRNA was isolated using the Poly(A)Pure kit (Ambion, Austin, TX). Northern blots were prepared using standard denaturing formaldehyde agarose electrophoresis techniques and transferred to GeneScreen Plus (NEN-Dupont, Boston, MA). The EST clone R84298 was obtained from the I.M.A.G.E. consortium (Lennon et al. (1996) Genomics 33, 151-152) through the ATCC. Both fetal liver cDNA libraries (λgt11 and Marathon cDNA) were obtained from Clontech (Palo Alto, CA). The J5DC T cell library was prepared from 48 hr PHA-activated T lymphocyte mRNA using the Superscript Choice system (Life Technologies Inc.) and ligated into pcDNAI/Amp (Invitrogen, Carlsbad, CA). The GF activated T cell library was prepared and ligated into pCDM8 as described previously [Hall et al. (1996) *Proc.*

Natl. Acad. Sci. USA 93, 11780-11785]. The expression vectors, pRc/CMV and pSecTag2B were obtained from Invitrogen. All labeling of DNA probes with ^{32}P-dCTP was by random priming (Life Technologies Inc.).

Isolation of cDNA encoding soluble attractin-1: Tryptic/chymotryptic peptides were prepared and analyzed as described previously [Duke-Cohan et al. (1995) *J. Biol. Chem.* 270, 14107-14114]. A multiple human hematopoietic tissue Northern blot (Clontech) was screened using the 1.2 kb MfeI/EcoNI R84298 EST. The λgt11-fetal liver library was screened using the 1.3 kb ClaI/HindIII R84298 EST which yielded the pks-43 (4kb) fragment. Hind III digestion of pks-43 released a 5′ 982 pb fragment which was used to rescreen the fetal liver library and a further 5′ sequence, including the putative start codon, was identified (pks-43-1). In order to produce full length recombinant soluble attractin-1, PCR-generated fragments encoding soluble attractin-1 were cloned into the expression vector, pRc/CMV. Using the pks-43-1 as template, a PCR (PCR1) was carried using the following primers: CCCAAGCTTGGGATGGGTGTCGGGCTCAGCCCGC - forward (SEQ ID NO:3) and, ATAAGAATGCGGCGCTAAACTCATTGTTCAGTTTCGACCTG - reverse (SEQ ID NO:4). A second PCR (PCR2) using the pks-43 fragment as template was carried out using the following primers: CCCAAGCTTGGGATGGTGGCCGCAGCGGCGGC - forward, (SEQ ID NO:5) and CCAGGTCCATCTGTCACAAACCCAG - reverse (SEQ ID NO:6). The fragment obtained from PCR1 was digested with HindIII and NaeI and that from PCR2 was digested with NaeI and NotI. The two digested fragments were then cloned together into HindIII/Not-I-digested pRc/CMV. For production of recombinant soluble attractin-1 with disabled start and stop codons into pSecTag2B, a 3.5 kb fragment was amplified from pRc/CMV-attractin using the Advantage GC cDNA PCR system (Clontech) and the primers

GTGCGTGAAGCTTGTACCGGCAACTGAGGCAAGGCTGA - forward (SEQ ID NO:7) and GTAGTTTTAAGTCCACGTTTGACTTCGCCGGCGTGCGTG - reverse (SEQ ID NO:8), digested with Hind II/Not I, and ligated into pSecTag2B.

Expression of functional soluble attractin-1: The Quick TnT system together with canine microsomal membranes (Promega, Madison, WI) was used for *in vitro* transcription and translation. 293T cells were transfected transiently using pSecTag2B-soluble attractin complexed with Lipofectamine Plus (Life Technologies Inc.) and assayed for soluble attractin-1 expression at 48 hr. For Western blotting experiments, cells were lysed in boiling SDS-PAGE sample buffer (x2) and samples run on SDS-PAGE gels, transferred to nitrocellulose by electroblotting, and the membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 1% BSA. Blots were incubated with murine antibody specific for myc (1:5000; Amersham, Arlington Heights, IL) or with a horseradish peroxidase (HRP) monoclonal antibody specific for myc (1:2000; Invitrogen) and detected using the Phototope chemiluminescent system (New England Biolabs). For immunoprecipitation experiments, the cells were solubilized in lysis buffer (1% Triton X-1000, 0.1% NP-40, 150 mM NaCl). Lysates were precleared with mIgG-agarose beads (Sigma) followed by incubation with Protein A-purified polyclonal rabbit antibody specific for soluble attractin or Protein A-purified normal preimmune IgG. Antibody complexes were isolated by incubation with agarose beads conjugated with antibody specific for rabbit IgG (Sigma) followed by boiling in 2x SDS-PAGE loading buffer, after which the procedure was identical with that described for Western transfers above.

For stable transfections, pSecTag2B-soluble attractin-1 was introduced into CHO cells by electroporation (250V, 1600 μF) using the Cell-Porator

apparatus (Life Technologies) and selection with zeocin (500 μg/ml; Invitrogen). To purify recombinant soluble attractin-1, cells were lysed and loaded onto a Talon Superflow metal affinity resin (Clontech) and eluted with 250 mM imidazole. DPPIV activity of the recombinant soluble attractin-1 was determined using gly-pro-pNA as substrate as described previously [Duke-Cohan et al. (1995) *J. Biol. Chem.* 270, 14107-14114].

Binding assays: PBMC were activated for 24 hr with PHA in AIM V medium as described above, washed in AIM V and 10^6 cells/100 μl were incubated for 1 hr at 4°C with doubling dilutions of ^{125}I-labeled soluble attractin [Duke-Cohan et al. (1996) *J. Immunol.* 156, 1714-1721], starting at 2 μg/ml (10^7 dpm/μg). The cells were washed with cold PBS, and the pellet and first wash supernatant were counted by scintillation. Results were analyzed by Scatchard analysis.

Electron microscopy: Cells were prepared as described previously [Xu et al. (1994) *J. Histochem. Cytochem.* 42, 1365-1376] and were analyzed by transmission electron microscopy (model JEM 100 CX II; JEOL, Peabody, MA).

Example 2. <u>Soluble Attractin-1 Binds Strongly to T Cells and Induces Spreading of Monocytes</u>.

Purified serum (soluble) attractin-1 had previously been found to enhance the proliferative responses of PBMC to recall antigens such as tetanus toxoid. In the absence of the antigen, the soluble attractin-1 had no effect. Scatchard analysis showed that about 1,000 molecules of soluble attractin-1 bound to the surface of a resting T cell, and about 2,000 molecules to a PHA-activated T cell, with a K_d for both of between 5 and 50 pM which is indicative of a specific, high affinity interaction. To determine whether the

binding of soluble attractin-1 had a functional effect upon cells, a population of PBMC was tested for morphological changes due to the presence or absence of soluble attractin-1. It was found that, within 48 hr of addition to PBMC in serum-free medium, soluble attractin-1 caused spreading of adherent macrophage-like cells, generating long processes to which lymphocytes attached. This process was dose-dependent, with the maximum effect occurring between 5 to 10 μg/ml soluble attractin-1 (Figs. 1A-1F). Soluble attractin-1 had no effect on E^+ lymphocytes alone (Fig. 1G) or E^- monocytes/B lymphocytes alone (Fig. 1H), but clustering occurred if the E^+ T and E^- populations were combined (Fig. 1I). After non-adherent cells had been washed away, the adherent cells were released by incubation with EDTA in PBS. By using immunofluorescence analysis, it was found that the small adherent cells were exclusively $CD3^+$ T cells, while the large adherent cells were predominantly $CD14^+$ monocytes/macrophages.

Example 3. <u>Cloning of cDNA Encoding Soluble Attractin-1 and Analysis of the Deduced Protein Structure</u>.

Peptide sequences within the soluble attractin-1 polypeptide were identified with a view to cloning cDNA encoding it. Natural soluble attractin-1 was purified to homogeneity and the N-termini of 16 proteolytic peptides (underlined in Fig. 2) were sequenced. One of the sequences (17 amino acids) was 100% identical to part of the derived amino acid sequence of a translated 3′ EST sequence (R84298) which codes for 1.9 kb sequence including the 3′ end of soluble attractin. By using this sequence as a probe, two mRNA species of 4.4 kb and 8-9 kb were detected, both of which were heavily represented in fetal liver and spleen (Fig. 3A). The larger form was dominant in thymus while the smaller form was dominant in

PBMC. No upregulation of attractin mRNA transcription was observed in activated T cells (Fig. 3B).

Based on mRNA expression, a fetal liver library was screened, and a 982 bp 5′ fragment derived from the longest clone (pks43) was used to rescreen the library, leading to identification of a further clone containing additional 5′ sequence (pks43-1). Sequencing of both overlapping clones yielded an open reading frame (ORF) of 3.594 kb that encoded all 16 peptides previously identified. PCR amplification of the main body of soluble attractin-1 cDNA from the two activated T cell libraries and from the fetal liver library produced PCR products of the same size, i.e., about 3 kb (Fig. 3C). The nucleotide sequence coding for soluble attractin-1 (SEQ ID NO:2) has been deposited in GenBank and given the accession number AF034957.

The codon encoding the first methionine of soluble attractin-1 is within a consensus Kozak sequence, and the subsequent ORF codes for a 134 kDa protein with 26 potential N-glycosylation sites. Although soluble attractin-1 is heavily glycosylated, no consensus leader sequence/signal peptides could be identified. Several distinct domains and motifs can be identified in the ORF, as depicted in Fig. 4B. These include a serine (Ser-26) within a hybrid of prolyl oligopeptidase and trypsin-like serine protease catalytic motifs (Fig. 4C), an EGF domain (Gly-24 to Gln-54), a CUB domain (His-57 to Phe-173), an EGF domain (Met-175 to Ala-207), the ligand-binding motif of the common γ cytokine chain (Cys-636 to Trp-648), a C-type lectin domain (Ile-713 to Cys-844), and 2 cysteine-rich regions incorporating the C-terminal laminin-like EGF domains (Ala-988 to Lys-1031 and Pro-1034 to Cys 1066). Fragments of soluble attractin-1 containing one or more of these domains are within the invention, as are nucleic acids encoding such fragments.

The only highly significant match at both the nucleotide and amino acid level (31% identity, 45% similarity across the complete 1198 amino acids) is with the nematode F33C8.1 perlecan-like protein, which has an identical organization of the CUB and EGF domains and conserved cysteine positioning suggestive of a similar secondary structure in the two proteins (Fig 4B). In contrast to soluble attractin-1, F33C8.1 does not express the γ chain binding motif or the C-type lectin domain. Soluble attractin-1 appears to be a new member of the CUB domain family of proteins, initially consisting of the complement proteins C1r/C1s, Uegf and BMP-1 [Bork et al. (1993) *Mol. Biol.* 231, 539-545].

Example 4. <u>Subcellular Localization and Synthesis of the Soluble Attractin Molecule</u>.

To understand the secretory route followed by soluble attractin-1 in the absence of a signal peptide, we determined the subcellular localization of soluble attractin-1 in resting and activated E^+ T lymphocytes. No glycosylated soluble attractin-1 could be detected anywhere in resting T cells (Fig. 5A), whereas in T cells activated for 48 hr with PHA, soluble attractin-1 was clearly localized in large vesicular structures (Fig. 5B) that often contained an electron-dense core (Fig. 5C). Vesicles containing soluble attractin-1 were often clustered close to the plasma membrane where they released soluble attractin-1 into the extracellular space (Fig. 5D). Soluble attractin-1-encoding cDNA was cloned into the expression vector pRc/CMV which was transfected in CHO cells. Glycosylated soluble attractin-1 could not be detected in the transfected CHO cells. The post-translational glycosylation of soluble attractin-1 was "forced" by cloning soluble attractin-1 cDNA into the pSecTag2B expression vector which supplies a N-terminal

leader sequence. In an *in vitro* transcription/translation system, pSecTag2B-soluble attractin-1 produced a protein of 134 kDa and in the presence of glycosyl transferases yielded a product of about 180 kDa (Fig. 6A). The correctly sized product was also detected by Western blotting, with antibody specific for myc, of a whole cell lysate of 293T cells transiently transfected with pSecTag2B-soluble attractin-1 (Fig. 6B). The polyclonal antibody specific for soluble attractin immunoprecipitated recombinant soluble attractin-1 from CHO cells stably transfected with pSecTag2B-attractin, confirming that the overall structure of the recombinant protein was similar to that of the purified natural material (Fig. 6C).

Example 5. <u>Functional Activities of Recombinant Soluble Attractin-1</u>.

Soluble attractin-1 was isolated from lysates of stably transfected CHO cells. Even with a signal peptide, the recombinant attractin localized intracellularly and was not secreted. The DPPIV enzyme activity of the recombinant protein was 0.42 units/mg, in comparison with 0.79 units/mg for T cell-released soluble attractin-1, 1.78 units/mg for serum attractin, and 4.12 units/mg for recombinant CD26.

The PBMC interaction assays depicted in Fig. 1 were repeated using recombinant rather than natural serum-purified soluble attractin-1. This experiment showed that the spreading effect of recombinant soluble attractin-1 on monocytes/macrophages was, as in the experiments with natural serum-purified soluble attractin-1, dose-dependent (Figs. 7A-7D). At a concentration of 5 μg/ml, the effect was similar to that of 5 μg/ml natural soluble attractin-1 and thus confirmed the results observed with purified natural soluble

attractin-1. There was a greater tendency for clustering of T cells when the recombinant soluble attractin-1 was used. However, these clusters were not proliferating cells, as indicated by the fact that no increase in $[^3H]$-thymidine incorporation could be detected over background in cultures of PBMC containing recombinant soluble attractin-1.

Example 6. <u>Evidence for Involvement of Attractin in Human Immune Responses</u>.

Clinical studies were performed in order to investigate whether attractin plays a role in the human immune system *in vivo*. Common variable immunodeficiency (CVI) is a late-onset primary immunodeficiency affecting either humoral or cellular immunity. The mechanism underlying this disease is still unknown. In a normal immune response, the surface expression of attractin is upregulated during T cell activation. The early signaling events during T cell activation were studied in 11 patients (age range: 7-27 years) affected by CVI to determine if the expression pattern of attractin is different from that of normal individuals. Cell-surface activation markers, including attractin, were evaluated on resting or 24-48 hour CD3-activated T cells by dual color fluorescence. In cells from all patients but one, in contrast to those from normal control subjects, the T cell surface expression of attractin was not upregulated after CD3 crosslinking. The lack of attractin upregulation was selective in that an increase of the other activation markers was observed in the CVI patients.

Example 7. Production of Single Chain Variable Region Fragment (scFv) Antibodies Specific for Attractin.

Initial efforts to produce murine monoclonal antibodies were unsuccessful, apparently as a direct consequence of the high degree of conservation between the mouse sequence identified to date and the human sequence for attractin. To overcome the problem of antigen conservation across species, techniques have been developed for cloning antigen-binding regions of V^H and V^L genes from the mRNA of non-immune spleen cells, connecting the variable regions with a flexible peptide linker to produce single chain variable region fragments (scFv). The scFv are then expressed as fusion proteins with phage coat protein [Sheets et al. (1998) *Proc. Natl. Acad. Sci. USA.* 95, 6157-6162] in phage particles. Using this technique, it is possible to generate repertoires of 10^7 to 10^{10} scFv variants. The reagents for generating a library and subsequent enrichment of murine antigen-specific scFv are now available in kit form from Amersham Pharmacia Biotech.

Three separate libraries are simultaneously produced from murine spleen cells. The first library is produced from mRNA of non-immune spleen cells, the second from spleen cells of mice which have received prior immunization with glycosylated native attractin, and a third library from spleen cells of mice which have been immunized with deglycosylated attractin. Messenger RNA is extracted from the lysed spleen cell populations by hybridization to oligo dT cellulose and first strand cDNA is synthesized using M-MuLV reverse transcriptase. Using primers designed to anneal to the 5′ and 3′ ends of murine heavy chain and kappa light chain variable region sequence, the repertoire of expressed variable regions sequences is amplified by PCR. Lambda light chain sequences are not amplified as they represent only a

small fraction of mature antibody-expressed light chain, and the flanking sequences are variable. The PCR products from the heavy chain amplification (≈400 bp) and the kappa light chain amplification (≈300 bp) are separated by agarose gel electrophoresis, gel plugs cut out, and the DNA extracted by freezing and thawing followed by membrane centrifugation.

A linker that hybridizes to the 3′ end of the heavy chain and the 5′ end of the light chain and encodes the peptide liner (e.g., Gly_4Ser_3) is then used to amplify out a full length scFv region of 750 bp. Through use of 5′ and 3′ primers which carry 5′ tails encoding restriction endonuclease sites, Sfi I and Not I sites are added by PCR to the 5′ and 3′ termini, respectively, of the scFv. After digestion with Sci I followed by Not I, the phenol/chloroform-precipitated fragments are directionally ligated into the pCANTAB 5E phagemid and the construct encoding multiple scFv is used to transform *E. coli* TG1 cells. The transformed cells are then infected with M13K07 helper phage to rescue the phagemid which will result in expression of the recombinant scFv as a fusion protein based on the gene III phage protein, the fusion protein being displayed on the phage particles.

At this point, the scFv-expressing phages are panned on attractin-coated plates, unbound phages are washed away, bound phage eluted, the phage DNA isolated, and TG1 cells reinfected and allowed to express by superinfection with helper phage. The panning process is repeated up to 3 times in order to refine the specificity. Once antigen-reactive clones have been identified, they are tested for binding by adding the scFv-expressing phage to attractin-coated wells, washing away unbound phage, adding horseradish peroxidase conjugated antibody specific for M13 phage, washing away

unbound conjugate and adding ABTS substrate. After confirmation of specific binding by ELISA, the recombinant phage is used to infect *E. coli* HB2151, which recognizes the amber stop codon encoded by pCANTAB 5E, allowing production of soluble scFv not in the context of the phage gene III protein. The soluble scFv can be isolated from *E. coli* periplasm using standard procedures and the sample can then be applied to an affinity column bearing antibody directed against a peptide region (-Gly-Ala-Pro-Val-Pro-Tyr-Pro-Asp-Pro-Leu-Glu-Pro-Arg-Ala-Ala-COOH (SEQ ID NO:9)) downstream from the kappa light chain and which generates a C-terminal common to all the scFv fragments. Binding occurs at neutral pH and, comparable to a normal antigen-antibody reaction, can be eluted at acidic pH. This peptide can be recognized by its specific antibody under native and denatured conditions. scFv produced in this way will provide a very specific handle for studying T lymphocyte intracellular expression and surface expression of attractin, for determining the kinetics of surface expression during clustering, for blocking functional clustering assays, and for determining both levels of attractin released from T cells during assays in vitro and levels in biological fluids.

In addition to maximizing selection of antibodies with the greatest affinity, panels of scFv directed against specific epitopes of attractin are developed. This is achieved by taking all isolated attractin-specific scFv and repeating the ELISA assays described above using the above-described forms of attractin and attractin deletion mutants as the target antigen. Selective binding of the scFv to one form of attractin but not to another or the deletion mutants will indicate that the scFv is specific for the relevant form of

attractin or the deleted part of the polypeptide, respectively.

Example 8. Identification of the Nucleotide and Amino Acid Sequence of Membrane Attractin-1

Initially, a 9 kb murine mRNA was shown to encode a molecule which was similar to human attractin-1. Sequence analysis of murine cDNA revealed that the nucleotide sequence diverged, extended the open reading frame, and coded for a transmembrane domain and a cytoplasmic domain. Given the 93% identity in the amino acid sequences of the regions shared by human soluble attractin-1 and murine attractin, it was hypothesized that an oligonucleotide probe based on the region coding for the murine cytoplasmic tail would also hybridize to a human mRNA that coded for an attractin with a cytoplasmic tail. This was found to be the case. Such a probe hybridized to the 9 kb human attractin mRNA but not to the 4.5 kb form, while a probe based on sequence coding for the common N-terminal sequence hybridized to both forms. This indicated that the 9kb human mRNA coded for a long attractin similar to the murine molecule. Searching of the EST database then revealed a deposited human sequence (KIAA0548; GenBank AB011120) that coded for 451 amino acids at the C-terminal of a human membrane attractin. The identification of this sequence re-enforced the hypothesis that a natural mRNA for membrane attractin existed. Using KIAA0548 as a base, a human genomic clone that contained an apparent attractin exon corresponding to the sequence coding for the amino acid sequence CEVENRYQGNPLRGTCY (SEQ ID NO:20), close to the C-terminal of attractin, was identified. Complete sequencing of this genomic clone proved conclusively that the divergence between soluble attractin-1, and membrane attractin-1, was the result of alternate splicing.

The nucleotide sequence of cDNA encoding human membrane attractin-1 (SEQ ID NO:11) is shown in Fig. 10 and the amino acid sequence of the human membrane attractin-1 protein (SEQ ID NO:10) is shown in Fig. 9. Note that the C-terminal five amino acids of soluble attractin-1 (SEQ ID NO:2 in Fig. 2) differs from the equivalent five amino acids in membrane attractin-1 and it lacks an additional 156 C-terminal amino acid residue region containing a transmembrane domain (amino acid residues 1205-1225) domain and a cytoplasmic domain C-terminal to the transmembrane domain.

Example 9. <u>Deduction of the Attractin-2 cDNA and Protein Sequences.</u>

Multiple attractin cDNA species covering the 5′ region have been identified which include or do not include a 222-bp insertion encoding a 74 amino acid region that defines attractin-2 proteins. Since there is no reason to suppose that this region can in any way influence 3′ splicing events, it is likely that both membrane and soluble attractin mRNA species containing the 222-bp insertion are generated. Further evidence that such transcripts exist comes from experiments with mouse mRNA from which it is clear that there are multiple mouse attractin mRNA species of which the membrane form, at least, contains a 72 amino acid (216 bp) insertion corresponding to the 74 amino acid insertion of human attractin-2. Thus, the invention includes both soluble attractin-2 (SEQ ID NO:18) (Fig. 11), membrane attractin-2 (SEQ ID NO:12) (Fig. 13) and the cDNA sequences encoding soluble attractin-2 (SEQ ID NO:19) (Fig. 12) and membrane attractin-2 (SEQ ID NO:13) (Fig. 14). The transmembrane domain of membrane attractin-2 is predicted to include residues 1279-1301 of SEQ ID NO:12 and its

cytoplasmic domain is predicted to include all residues C-terminal of the transmembrane domain.

Example 10. Recombinant Soluble Attractin-1 Increases Expression of MHC Class II and B7.1 Molecules on Monocytes.

Peripheral blood leukocytes were isolated (from blood donated by normal donors in the Blood Bank of the Dana Farber Cancer Institute) by centrifugation over Ficoll-Hypaque. The isolated cells were suspended in defined lymphocyte culture medium (AIM V; GIBCO-BRL) at a concentration of $2x10^6$ per ml and incubated at 37°C in an atmosphere of 7.5% CO_2 for 72 hr with soluble recombinant attractin-1 (at 5 ug/ml) or with GM-CSF (10 U/ml). Non-adherent cells were then discarded and adherent cells were recovered using PBS containing 5 mM EDTA and washed in AIM V medium. All cells were suspended at a concentration of 10^7 per ml. 100 ul aliquots (corresponding to 10^6 cells) were incubated with 0.25 to 1 ug of a panel of FITC- or phycoerythrin-labelled antibodies directed against a selection of known pan-leukocyte, T cell-specific, B cell-specific, monocyte-specific and NK-specific markers. Using the Coulter XL fluorescence analysis machine, fluorescence windows were gated on the total cell population, the lymphocyte population, and the monocyte population that is distinguished by size and light "scatter" (Fig. 15). The profiles with dark fill were obtained with control cells incubated with antibodies of irrelevant specificity conjugated with appropriate fluorophores, and those with light fill were obtained with cells incubated with antibodies with the indicated specificity. Also shown in Fig. 15 are similar data obtained with peripheral blood leukocytes prior to culture (DO).

Monocytes incubated with GM-CSF differentiated by losing CD14 (Fig. 15) and expressing CD1a (not shown), indicating a differentiation towards early dendritic cells. For monocytes incubated with soluble attractin-1 in the presence of CD3+ T lymphocytes, CD14 remains high, there is no induction of CD1a (not shown), B7-1 expression begins to increase, and MHC Class II expression increases off scale (Fig. 15). These findings indicate that attractin serves to enhance the antigen presenting function of monocytes.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. An isolated DNA comprising:

(a) a nucleic acid sequence that encodes a polypeptide that enhances spreading of a macrophage or a monocyte and that hybridizes under highly stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18; or

(b) the complement of the nucleic acid sequence.

2. The DNA of claim 1, wherein the nucleic acid sequence encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18.

3. The DNA of claim 1, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:11, SEQ ID NO:13, and SEQ ID NO:19.

4. An isolated polypeptide comprising:

an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18; or differing from SEQ ID NO: 10, 12, or 18 solely by one or more conservative amino acid substitutions.

5. A fusion protein comprising:

(a) an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18, but lacking methionine at position 1 of said amino acid sequence; and

(b) a heterologous leader peptide.

6. An isolated nucleic acid encoding the fusion protein of claim 5.

7. A method of enhancing spreading of a macrophage or a monocyte *in vitro*, the method comprising co-culturing a T cell and a monocyte or a macrophage with an agent selected from the group consisting of:

(a) an isolated attractin polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18;

(b) a functional fragment of the attractin polypeptide; and

(c) the polypeptide or the functional fragment, but with at least one conservative amino acid substitution.

8. A method of treating a mammal in need of an enhanced immune response, the method comprising delivering to a tissue of the mammal that contains T cells and macrophages or monocytes, an agent selected from the group consisting of:

(a) an isolated attractin polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18;

(b) a functional fragment of the attractin polypeptide; and

(c) the polypeptide or the functional fragment, but with at least one conservative amino acid substitution.

9. The method of claim 8, wherein the delivery comprises administration of the agent to the mammal.

10. The method of claim 8, wherein the delivery comprises administering to the mammal a nucleic acid encoding the agent.

11. The method of claim 8, wherein the mammal is a human.

12. The method of claim 11, wherein the human is suspected of being immunodeficient.

13. The method of claim 11, wherein the human is suspected of having cancer.

14. The method of claim 13, wherein the method is performed before, during, or after chemotherapy or radiation therapy.

15. A method of inhibiting spreading of a macrophage or a monocyte in a mammal, the method comprising administering to the mammal an isolated compound that binds to an attractin polypeptide.

16. The method of claim 15, wherein the compound is an antibody.

17. The method of claim 15, wherein the mammal is a human.

18. The method of claim 17, wherein the human is suspected of having an autoimmune disease.

19. The method of claim 17, wherein the human is a transplant recipient.

20. A vector comprising the isolated DNA of claim 1.

21. The vector of claim 20, wherein the nucleic acid sequence is operably linked to a regulatory element which allows expression of said nucleic acid in a cell.

22. A cultured cell comprising the vector of claim 21.

23. A method of producing a polypeptide, the method comprising culturing the cell of claim 22 and purifying the polypeptide from the cell.

24. A vector comprising the isolated nucleic acid of claim 6.

25. The vector of claim 24, wherein the nucleic acid is operably linked to a regulatory element which allows expression of said nucleic acid in a cell.

26. A cell comprising the vector of claim 25.

27. A method of producing a fusion protein, the method comprising culturing the cell of claim 26 and purifying the fusion protein from the cell.

28. A method of identifying a compound that inhibits an immune response, the method comprising:

a) providing an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18, or the amino acid sequence but with one or more conservative amino acid substitutions;

b) co-culturing a T cell and a macrophage or a monocyte with the isolated polypeptide and the test compound;

c) determining whether the test compound inhibits spreading of the macrophage or the monocyte, as an indication that the test compound inhibits an immune response.

29. A method of identifying a compound that enhances an immune response, the method comprising:

a) providing a test compound;

b) combining the test compound, a T cell, a macrophage or a monocyte, and an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18, or the amino acid sequence with one or more conservative substitutions; and

c) determining whether the test compound enhances spreading of the macrophage or the monocyte, as an indication that the test compound inhibits an immune response.

30. An antibody that binds to a polypeptide selected from the group consisting of SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18, but that does not bind to CD26 or to a polypeptide with the sequence of SEQ ID NO:2.

31. The antibody of claim 30, wherein the antibody is a single chain variable region fragment (scFv).

32. A method of treating a mammal in need of an enhanced immune response, the method comprising:

a) providing a recombinant cell which is the progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding an agent or a functional fragment of the agent so that the cell expresses the agent or functional fragment; and

b) administering the cell to the mammal,

wherein the agent is selected from the group consisting of:

(i) an attractin polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:18;

(ii) a functional fragment of the attractin polypeptide; and

(iii) the polypeptide or the functional fragment, but with one or more conservative amino acid substitutions.

33. An isolated functional attractin fragment comprising amino acid residues 31-104 of SEQ ID NO:12 or SEQ ID NO:18.

34. An isolated functional attractin fragment comprising amino acid residues 1279-1301 of SEQ ID NO:12.

35. The isolated functional attractin fragment of claim 34, comprising amino acid residues 1219-1429 of SEQ ID NO:12.

36. An isolated functional attractin fragment comprising amino acid residues 1302-1429 of SEQ ID NO:12.

37. The method of claim 12, wherein said human is suspected of having common variable immunodeficiency syndrome.

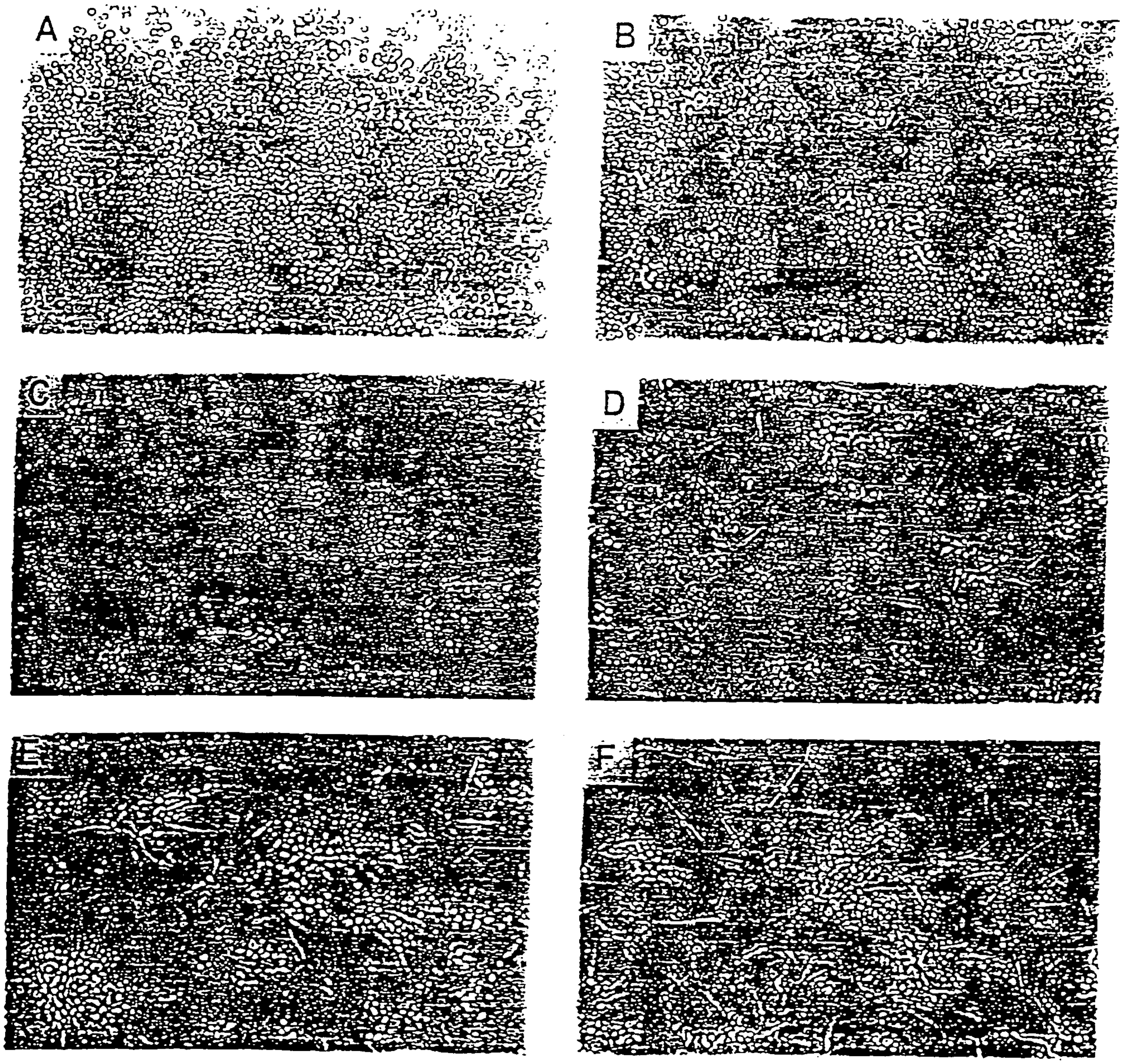

Fig 1A — 1F

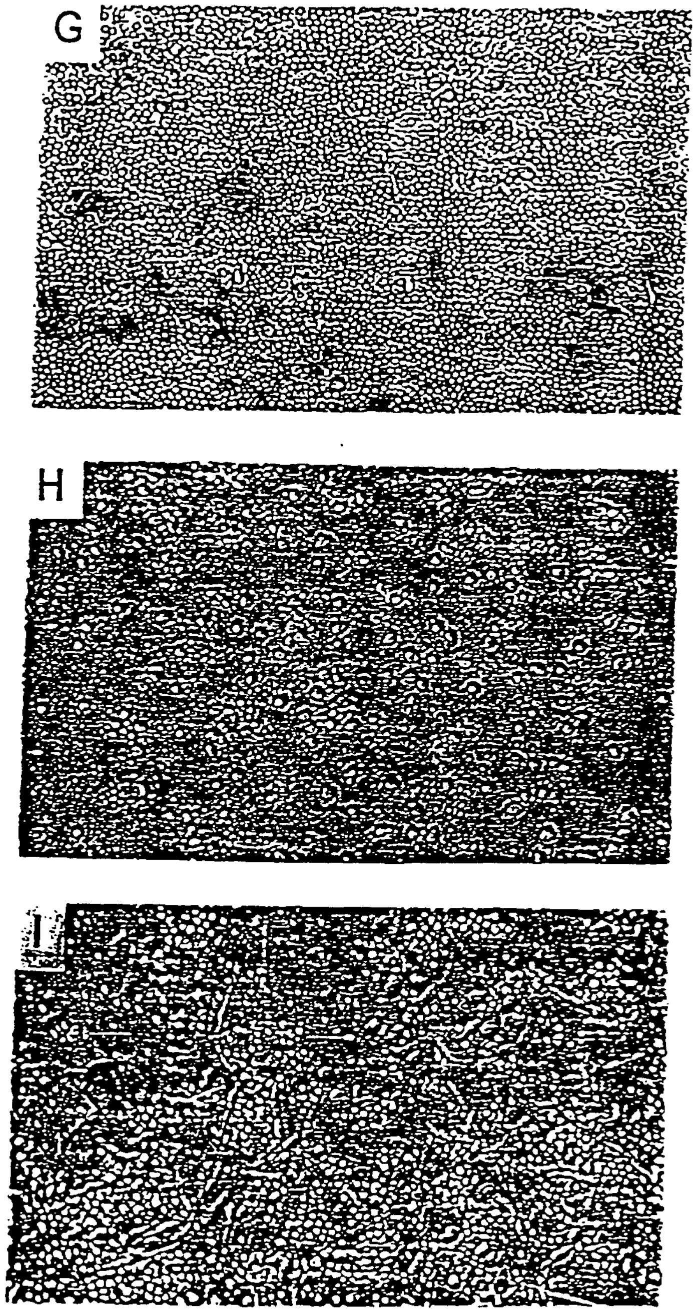

Fig 1G - 1I

1 MVAAAAATEA RLRRRTAATA ALAGRSGGPH CVNGGRCNPG TGQCVCPAGW
51 VGEQCQHCGG RFRLTGSSGF VTDGPGNYKY KTKCTWLIEG QPNRIMRLRF
101 NHFATECSWD HLYVYDGDSI YAPLVAAFSG LIVPERDGNE TVPEVVATSG
151 YALLHFFSDA AYNLTGFNIT YSFDMCPNNC SGRGECKISN SSETVECECS
201 ENWKGEACDI PHCTDNCGFP HRGICNSSDV RGCSCFSDWQ GPGCSVPVPA
251 NQSFWTREEY SNLKLPRASH KAVVNGNIMW VVGGYMFNHS DYNMVLAYDL
301 ASREWLPLNR SVNNVVVRYG HSLALYKDKI YMYGGKIDPT GNVTNELRVF
351 HIHNESWVLL TPKAKEQYAV VGHSAHIVTL KNGRVVMLVI FGHCPLYGYI
401 SNVQEYDLDK NTWSILHTQG ALVQGGYGHS SVYDHRTRAL YVHGGYKAFS
451 ANKYRLADDL YRYDVDTQMW TILKDSRFFR YLHTAVIVSG TMLVFGGNTH
501 NDTSMSHGAK CFSSDFMAYD IACDRWSVLP RPDLHHDVNR FGHSAVLHNS
551 TMYVFGGFNS LLLSDILVFT SEQCDAHRSE AACLAAGPGI RCVWNTGSSQ
601 CISWALATDE QEEKLKSECF SKRTLDHDRC DQHTDCYSCT ANTNDCHWCN
651 DHCVPRNHSC SEGQISIFRY ENCPKDNPMY YCNKKTSCRS CALDQNCQWE
701 PRNQECIALP ENICGIGWHL VGNSCLKITT AKENYDNAKL FCRNHNALLA
751 SLTTQKKVEF VLKQLRIMQS SQSMSKLTLT PWVGLRKINV SYWCWEDMSP
801 FTNSLLQWMP SEPSDAGFCG ILSEPSTRGL KAATCINPLN GSVCERPANH
851 SAKQCRTPCA LRTACGDCTS GSSECMWCSN MKQCVDSNAY VASFPFGQCM
901 EWYTMSTCPP ENCSGYCTCS HCLEQPGCGW CTDPSNTGKG KCIEGSYKGP
951 VKMPSQAPTG NFYPQPLLNS SMCLEDSRYN WSFIHCPACQ CNGHSKCINQ
1001 SICEKCENLT TGKHCETCIS GFYGDPTNGG KCQPCKCNGH ASLCNTNTGK
1051 CFCTTKGVKG DECQLCEVEN RYQGNPLRGT CYYTLLIDYQ FTFSLSQEDD
1101 RYYTAINFVA TPDEQNRDLD MFINASKNFN LNITWAASFS AGTQAGEEMP
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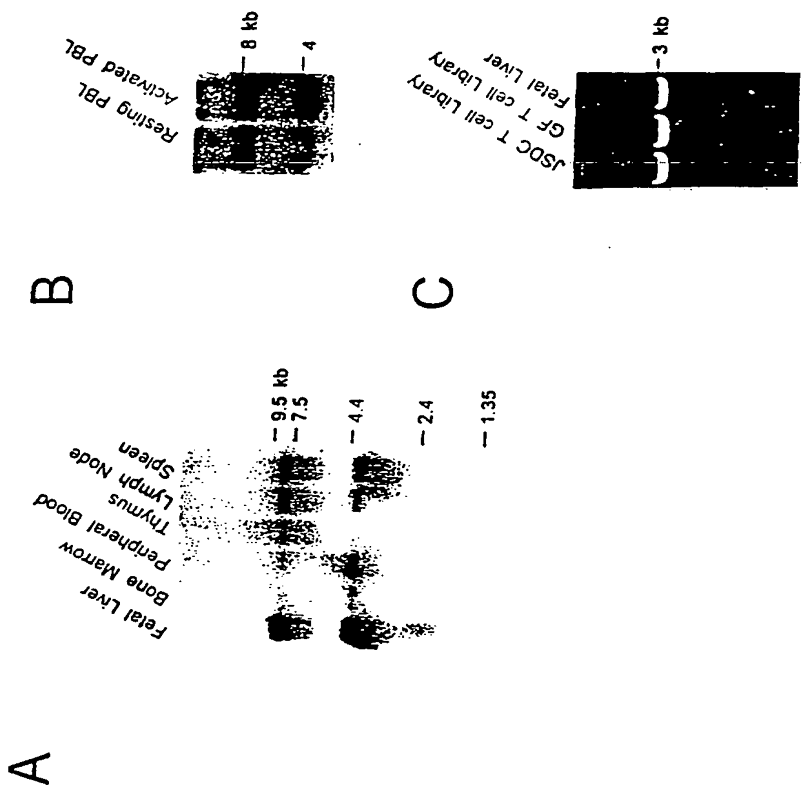
Fig. 2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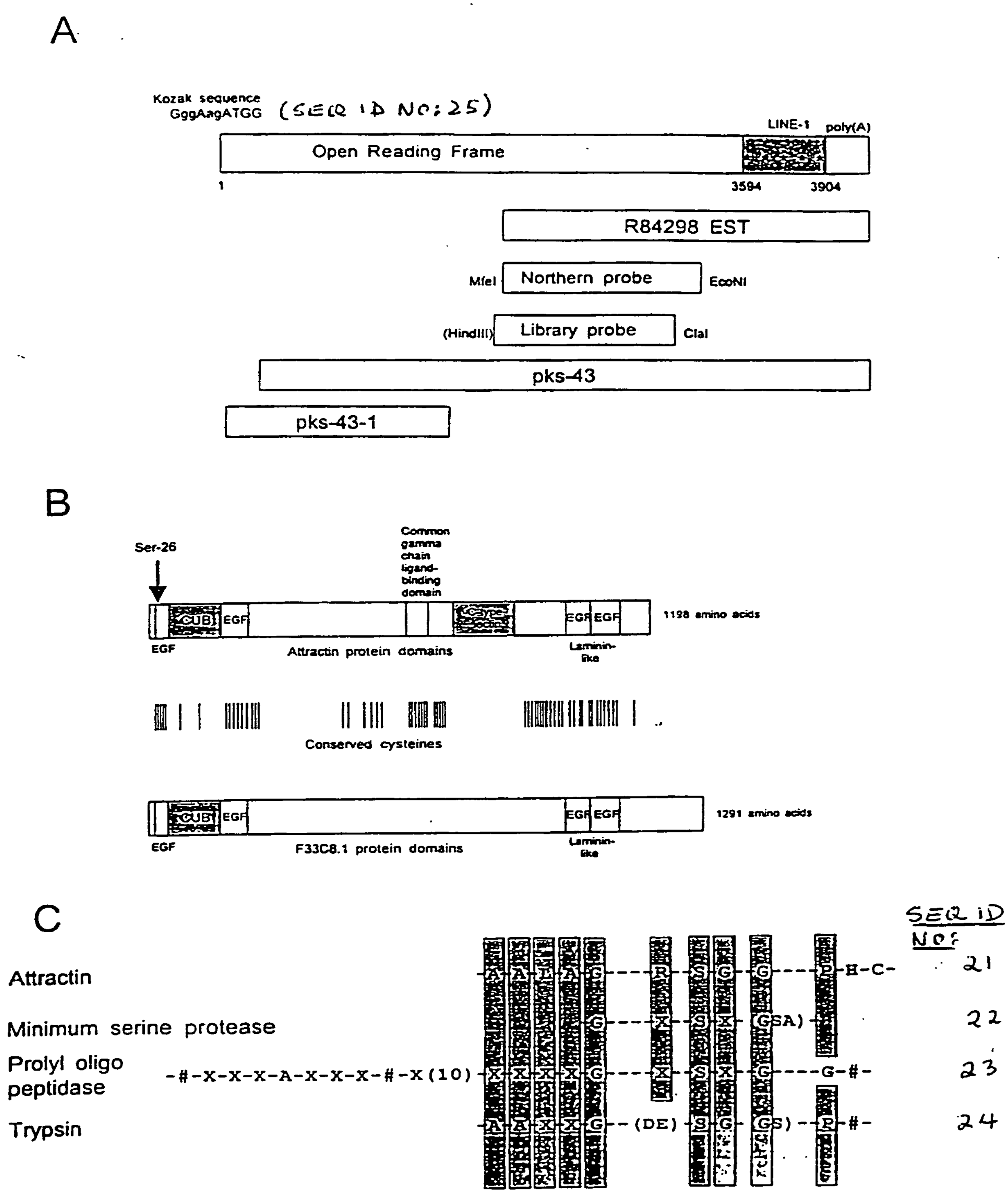
3 kb

Fig. 3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EcoNI
(HindIII)
Library probe
ClaI
pks-43
pks-43-1
B
Ser-26
Common gamma chain ligand-binding domain
CUB
EGF
C-type lectin
EGF EGF
1198 amino acids
EGF
Attractin protein domains
Laminin-like
Conserved cysteines
CUB
EGF
EGF EGF
1291 amino acids
EGF
F33C8.1 protein domains
Laminin-like
C
SEQ ID NO:
Attractin
-A-A-L-A-G---R-S-G-G----P-H-C-
21
Minimum serine protease
-G---X-S-X-GSA)
22
Prolyl oligo peptidase
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23
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24

Fig. 4

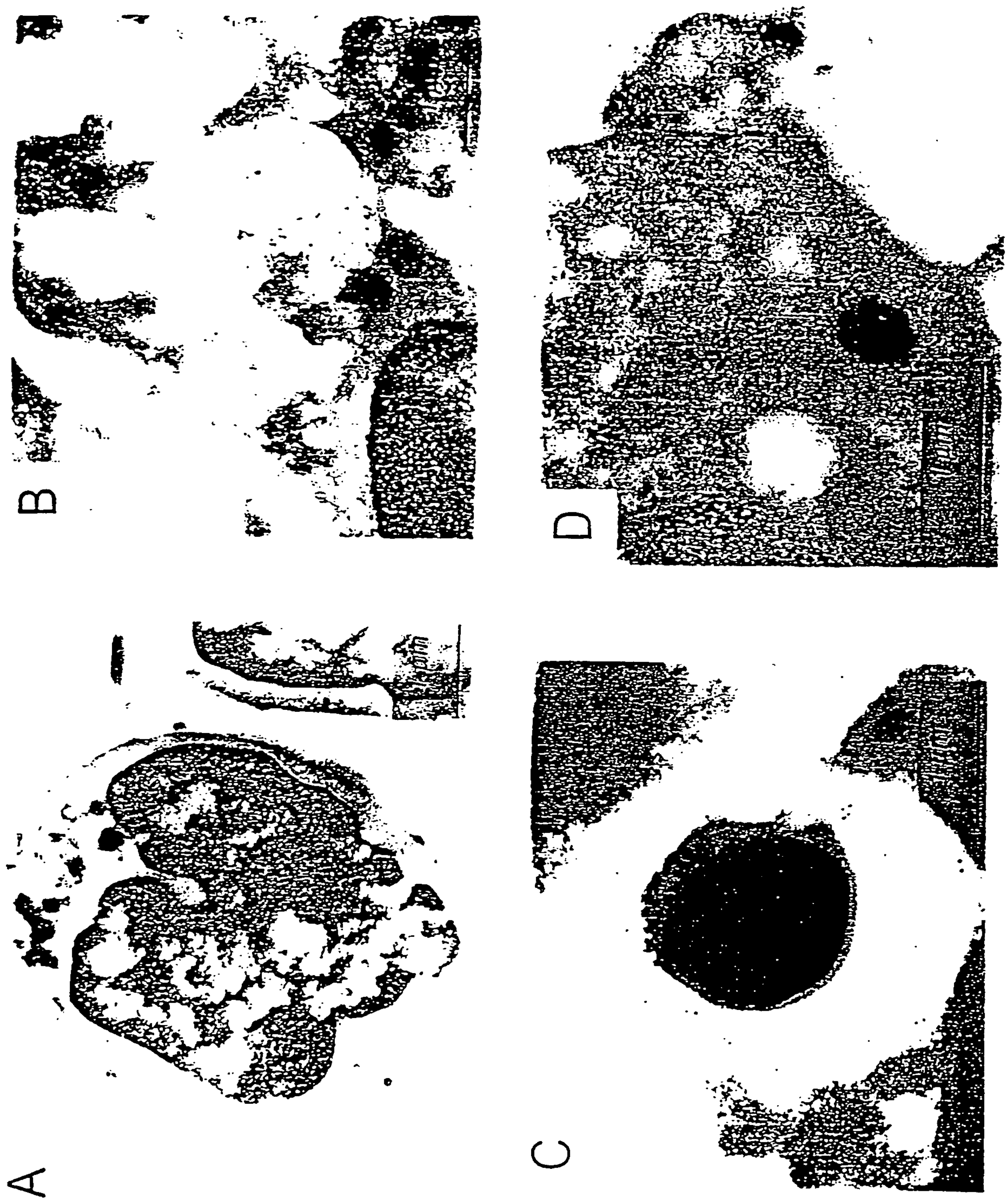
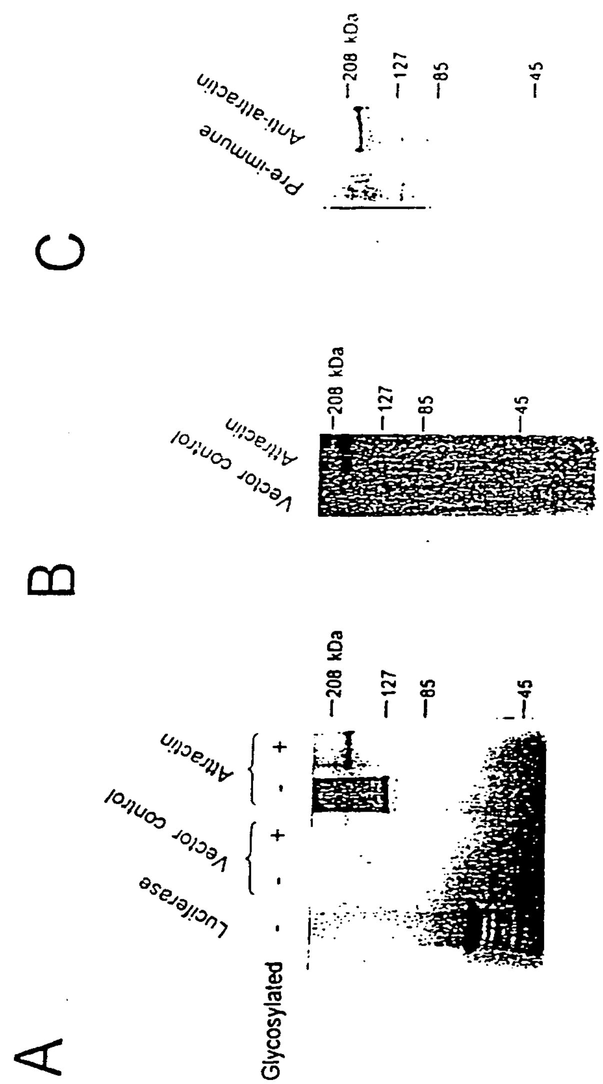


Fig. 5


A
B
C
Glycosylated
Luciferase
Vector control
Attracin
Vector control
Attracin
Pre-immune
Anti-attracin
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—85
—45

Fig. 6

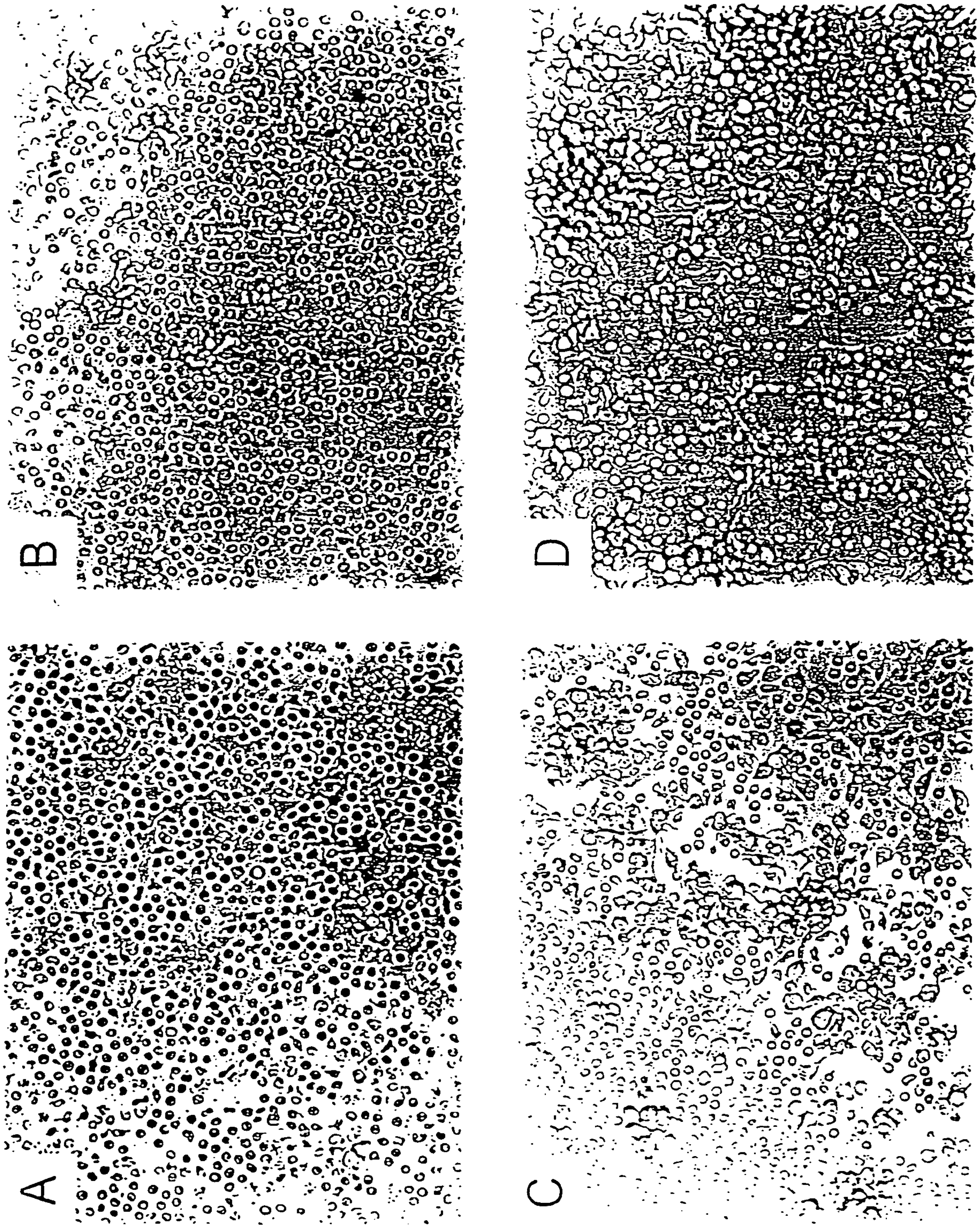

Fig. 7

1 ATGGTGGCCG CAGCGGCGGC AACTGAGGCA AGGCTGAGGA GGAGGACGGC

51 GGCGACGGCA GCGCTCGCGG GCAGGAGCGG CGGGCCGCAC TGTGTCAACG

101 GCGGTCGCTG CAACCCTGGC ACCGGCCAGT GCGTCTGCCC CGCCGGCTGG

151 GTGGGCGAGC AATGCCAGCA CTGCGGGGGC CGCTTCAGAC TAACTGGATC

201 TTCTGGGTTT GTGACAGATG GACCTGGAAA TTATAAATAC AAAACGAAGT

251 GCACGTGGCT CATTGAAGGA CAGCCAAATA GAATAATGAG ACTTCGTTTC

301 AATCATTTTG CTACAGAGTG TAGTTGGGAC CATTTATATG TTTATGATGG

351 GGACTCAATT TATGCACCGC TAGTTGCTGC ATTTAGTGGC CTCATTGTTC

401 CTGAGAGAGA TGGCAATGAG ACTGTCCCTG AGGTTGTTGC CACATCAGGT

451 TATGCCTTGC TGCATTTTTT TAGTGATGCT GCTTATAATT TGACTGGATT

501 TAATATTACT TACAGTTTTG ATATGTGTcC AAATAACTGC TCAGGcCGAG

551 GAGAGTGTAA GATCAGTAAT AGCAGCGAAA CTGTTGAATG TGAATGTTCT

601 GAAAACTGGA AAGGTGAAGC ATGTGACATT CCTCACTGTA CAGACAACTG

651 TGGTTTTCCT CATCGAGGCA TCTGCAATTC AAGTGATGTC AGAGGATGCT

701 CCTGCTTCTC AGACTGGCAG GGTCCTGGAT GTTCAGTTCC TGTACCAGCT

751 AACCAGTCAT TTTGGACTCG AGAGGAATAT TCTAACTTAA AGCTCCCCAG

801 AGCATCTCAT AAAGCTGTGG TCAATGGAAA CATTATGTGG GTTGTTGGAG

851 GATATATGTT CAACCACTCA GATTATAACA TGGTTCTAGC GTATGACCTT

901 GCTTCTAGGG AGTGGCTTCC ACTAAACCGT TCTGTGAACA ATGTGGTTGT

951 TAGATATGGT CATTCTTTGG CATTATACAA GGATAAAATT TACATGTATG

1001 GAGGAAAAAT TGATcCAACT GGGAATGTGA CCAATGAGTT GAGAGTTTTT

1051 CACATTCATA ATGAGTCATG GGTGTTGTTG ACCCCTAAGG CAAAGGAGCA

1101 GTATGCAGTG GTTGGGCACT CTGCACACAT TGTTACACTG AAGAATGGCC

1151 GAGTGGTCAT GCTGGTCATC TTTGGTCACT GCCCTCTCTA TGGATATATA

1201 AGCAATGTGC AGGAATATGA TTTGGATAAG AACACATGGA GTATATTACA

1251 CACCCAGGGT GCCCTTGTGC AAGGGGGTTA CGGCCATAGC AGTGTTTACG

1301 ACCATAGGAC CAGGGCCCTA TACGTTCATG GTGGCTACAA GGCTTTCAGT

Fig. 8A

1351 GCCAATAAGT ACCGGCTTGC AGATGATCTC TACCGATATG ATGTGGATAC
1401 CCAGATGTGG ACCATTCTTA AGGACAGCCG ATTTTTCCGT TACTTGCACA
1451 CAGCTGTGAT AGTGAGTGGA ACCATGCTGG TGTTTGGGGG AAACACACAC
1501 AATGACACAT CTATGAGCCA TGGCGCCAAA TGCTTCTCTT CAGATTTCAT
1551 GGCCTATGAC ATTGCCTGTG ACCGCTGGTC AGTGCTTCCC AGACCTGATc
1601 TCCACCATGA TGTCAACAGA TTTGGCCATT CAGCAGTCTT ACACAACAGC
1651 ACCATGTATG TGTTCGGTGG TTTCAATAGT CTCCTCCTCA GCGACATCCT
1701 GGTATTCACC TCGGAACAGT GTGATGCGCA TCGGAGTGAA GCCGCTTGTT
1751 TAGCAGCAGG ACCTGGTATT CGGTGTGTGT GGAACACAGG GTCGTCTCAG
1801 TGTATCTCGT GGGCGCTGGC AACTGATGAA CAAGAAGAAA AGTTAAAATC
1851 AGAATGTTTT TCCAAAAGAA CTCTTGACCA TGACAGATGT GACCAGCACA
1901 CAGATTGTTA CAGCTGtACA GCCAACACCA ATGACTGCCA CTGGTGCAAT
1951 GACCATTGTG TCCCCAGGAA CCACAGCTGC TCAGAAGGCC AGATCTCCAT
2001 TTTTAGGTAT GAGAATTGCC CCAAGGATAA CCCcATGTAC TACTGTAACA
2051 AGAAGACCAG CTGCAGGAGC TGTGCCCTGG ACCAGAACTG CCAGTGGGAG
2101 CCCCGGAATC AGGAGTGCAT TGCCCTGCCC GAAAATATCT GTGGCATTGG
2151 CTGGCATTTG GTTGGAAACT CATGTTTGAA AATTACTACT GCCAAGGAGA
2201 ATTATGACAA TGCTAAATTG TTCTGTAGGA ACCACAATGC CCTTTTGGCT
2251 TCTCTTACAA CCCAGAAGAA GGTAGAATTT GTCCTTAAGC AGCTGCGAAT
2301 AATGCAGTCA TCTCAGAGCA TGTCCAAGCT CACCTTAACC CCATGGGTCG
2351 GCCTTCGGAA GATCAATGTG TCCTACTGGT GCTGGGAAGA TATGTCCCCA
2401 TTTACAAATA GTTTACTACA GTGGATGCCG TCTGAGCCCA GTGATGCTGG
2451 ATTCTGTGGA ATTTTATCAG AACCCAGTAC TCGGGGACTG AAGGCTGCAA
2501 CCTGCATCAA CCCACTCAAT GGTAGTGTCT GTGAAAGGCC TGCAAACCAC
2551 AGTGCTAAGC AGTGCCGGAC ACCATGTGCC TTGAGGACAG CATGTGGAGA
2601 TTGCACCAGC GGCAGCTCTG AGTGCATGTG GTGCAGCAAC ATGAAGCAGT
2651 GTGTGGACTC CAATGCCTAT GTGGCCTCCT TCCCTTTTGG CCAGTGTATG

Fig. 8B

2701 GAATGGTATA CGATGAGCAC CTGCCCCCCT GAAAATTGTT CAGGCTACTG
2751 TACCTGTAGT CATTGCTTGG AGCAACCAGG CTGTGGCTGG TGTACTGATC
2801 CCAGCAATAC TGGCAAAGGG AAATGCATAG AGGGTTCCTA TAAAGGACCA
2851 GTGAAGATGC CTTCGCAAGC CCCTACAGGA AATTTCTATC CACAGCCCCT
2901 GCTCAATTCC AGCATGTGTC TAGAGGACAG CAGATACAAC TGGTCTTTCA
2951 TTCACTGTCC AGCTTGCCAA TGCAACGGCC ACAGTAAATG CATCAATCAG
3001 AGCATCTGTG AGAAGTGTGA GAACCTGACC ACAGGCAAGC ACTGCGAGAC
3051 CTGCATATCT GGCTTCTACG GTGATCCCAC CAATGGAGGG AAATGTCAGC
3101 CATGCAAGTG CAATGGGCAC GCGTCTCTGT GCAACACCAA CACGGGCAAG
3151 TGCTTCTGCA CCACCAAGGG CGTCAAGGGG GACGAGTGCC AGCTATGTGA
3201 GGTAGAAAAT CGATACCAAG GAAACCCTCT CAGAGGAACA TGTTATTATA
3251 CTCTTCTTAT TGACTATCAG TTCACCTTTA GTCTATCCCA GGAAGATGAT
3301 CGCTATTACA CAGCTATCAA TTTTGTGGCT ACTCCTGACG AACAAAACAG
3351 GGATTTGGAC ATGTTCATCA ATGCCTCCAA GAATTTCAAC CTCAACATCA
3401 CCTGGGCTGC CAGTTTCTCA GCTGGAACCC AGGCTGGAGA AGAGATGCCT
3451 GTTGTTTCAA AAACCAACAT TAAGGAGTAC AAAGATAGTT TCTCTAATGA
3501 GAAGTTTGAT TTTCGCAACC ACCCAAATAT CACTTTCTTT GTTTATGTCA
3551 GTAATTTCAC CTGGCCCATC AAAATTCAGG TGCAAACTGA ACAATGA

Fig. 8C

FIGURE 9

1	MVAAAAATEA	RLRRRTAATA	ALAGRSGGPH	CVNGGRCNPG	TGQCVCPAGW
51	VGEQCQHCGG	RFRLTGSSGF	VTDGPGNYKY	KTKCTWLIEG	QPNRIMRLRF
101	NHFATECSWD	HLYVYDGDSI	YAPLVAAFSG	LIVPERDGNE	TVPEVVATSG
151	YALLHFFSDA	AYNLTGFNIT	YSFDMCPNNC	SGRGECKISN	SSDTVECECS
201	ENWKGEACDI	PHCTDNCGFP	HRGICNSSDV	RGCSCFSDWQ	GPGCSVPVPA
251	NQSFWTREEY	SNLKLPRASH	KAVVNGNIMW	VVGGYMFNHS	DYNMVLAYDL
301	ASREWLPLNR	SVNNVVVRYG	HSLALYKDKI	YMYGGKIDST	GNVTNELRVF
351	HIHNESWVLL	TPKAKEQYAV	VGHSAHIVTL	KNGRVVMLVI	FGHCPLYGYI
401	SNVQEYDLDK	NTWSILHTQG	ALVQGGYGHS	SVYDHRTRAL	YVHGGYKAFS
451	ANKYRLADDL	YRYDVDTQMW	TILKDSRFFR	YLHTAVIVSG	TMLVFGGNTH
501	NDTSMSHGAK	CFSSDFMAYD	IACDRWSVLP	RPDLHHDVNR	FGHSAVLHNS
551	TMYVFGGFNS	LLLSDILVFT	SEQCDAHRSE	AACLAAGPGI	RCVWNTGSSQ
601	CISWALATDE	QEEKLKSECF	SKRTLDHDRC	DQHTDCYSCT	ANTNDCHWCN
651	DHCVPRNHSC	SEGQISIFRY	ENCPKDNPMY	YCNKKTSCRS	CALDQNCQWE
701	PRNQECIALP	ENICGIGWHL	VGNSCLKITT	AKENYDNAKL	FCRNHNALLA
751	SLTTQKKVEF	VLKQLRIMQS	SQSMSKLTLT	PWVGLRKINV	SYWCWEDMSP
801	FTNSLLQWMP	SEPSDAGFCG	ILSEPSTRGL	KAATCINPLN	GSVCERPANH
851	SAKQCRTPCA	LRTACGDCTS	GSSECMWCSN	MKQCVDSNAY	VASFPFGQCM
901	EWYTMSTCPP	ENCSGYCTCS	HCLEQPGCGW	CTDPSNTGKG	KCIEGSYKGP
951	VKMPSQAPTG	NFYPQPLLNS	SMCLEDSRYN	WSFIHCPACQ	CNGHSKCINQ
1001	SICEKCENLT	TGKHCETCIS	GFYGDPTNGG	KCQPCKCNGH	ASLCNTNTGK
1051	CFCTTKGVKG	DECQLCEVEN	RYQGNPLRGT	CYYTLLIDYQ	FTFSLSQEDD
1101	RYYTAINFVA	TPDEQNRDLD	MFINASKNFN	LNITWAASFS	AGTQAGEEMP
1151	VVSKTNIKEY	KDSFSNEKFD	FRNHPNITFF	VYVSNFTWPI	KIQIAFSQHS
1201	NFMDLVQFFV	TFFSCFLSLL	LVAAVVWKIK	QSCWASRRRE	QLLREMQQMA
1251	SRPFASVNVA	LETDEEPPDL	IGGSIKTVPK	PIALEPCFGN	KAAVLSVFVR
1301	LPRGLGGIPP	PGQSGLAVAS	ALVDISQQMP	IVYKEKSGAV	RNRKQQPPAQ
1351	PGTCI				

FIGURE 10A

```
1     atggtggccg cagcggcggc aactgaggca aggctgagga ggaggacggc
51    ggcgacggca gcgctcgcgg gcaggagcgg cgggccgcac tgtgtcaacg
101   gcggtcgctg caaccctggc accggccagt gcgtctgccc cgccggctgg
151   gtgggcgagc aatgccagca ctgcgggggc cgcttcagac taactggatc
201   ttctgggttt gtgacagatg gacctggaaa ttataaatac aaaacgaagt
251   gcacgtggct cattgaagga cagccaaata gaataatgag acttcgtttc
301   aatcatttg ctacagagtg tagttgggac catttatatg tttatgatgg
351   ggactcaatt tatgcaccgc tagttgctgc atttagtggc ctcattgttc
401   ctgagagaga tggcaatgag actgtccctg aggttgttgc cacatcaggt
451   tatgccttgc tgcatttttt tagtgatgct gcttataatt tgactggatt
501   taatattact tacagttttg atatgtgtcc aaataactgc tcaggccgag
551   gagagtgtaa gatcagtaat agcagcgata ctgttgaatg tgaatgttct
601   gaaaactgga aaggtgaagc atgtgacatt cctcactgta cagacaactg
651   tggttttcct catcgaggca tctgcaattc aagtgatgtc agaggatgct
701   cctgcttctc agactggcag ggtcctggat gttcagttcc tgtaccagct
751   aaccagtcat tttggactcg agaggaatat tctaacttaa agctccccag
801   agcatctcat aaagctgtgg tcaatggaaa cattatgtgg gttgttggag
851   gatatatgtt caaccactca gattataaca tggttctagc gtatgacctt
901   gcttctaggg agtggcttcc actaaaccgt tctgtgaaca atgtggttgt
951   tagatatggt cattctttgg cattatacaa ggataaaatt tacatgtatg
1001  gaggaaaaat tgattcaact gggaatgtga ccaatgagtt gagagttttt
1051  cacattcata atgagtcatg ggtgttgttg acccctaagg caaaggagca
1101  gtatgcagtg gttgggcact ctgcacacat tgttacactg aagaatggcc
1151  gagtggtcat gctggtcatc tttggtcact gccctctcta tggatatata
1201  agcaatgtgc aggaatatga tttggataag aacacatgga gtatattaca
1251  cacccagggt gcccttgtgc aagggggtta cggccatagc agtgtttacg
1301  accataggac cagggcccta tacgttcatg gtggctacaa ggctttcagt
1351  gccaataagt accggcttgc agatgatctc taccgatatg atgtggatac
1401  ccagatgtgg accattctta aggacagccg atttttccgt tacttgcaca
1451  cagctgtgat agtgagtgga accatgctgg tgtttggggg aaacacacac
1501  aatgacacat ctatgagcca tggcgccaaa tgcttctctt cagatttcat
1551  ggcctatgac attgcctgtg accgctggtc agtgcttccc agacctgatc
1601  tccaccatga tgtcaacaga tttggccatt cagcagtctt acacaacagc
1651  accatgtatg tgttcggtgg tttcaatagt ctcctcctca gcgacatcct
1701  ggtattcacc tcggaacagt gtgatgcgca tcggagtgaa gccgcttgtt
1751  tagcagcagg acctggtatt cggtgtgtgt ggaacacagg gtcgtctcag
1801  tgtatctcgt gggcgctggc aactgatgaa caagaagaaa agttaaaatc
1851  agaatgtttt tccaaaagaa ctcttgacca tgacagatgt gaccagcaca
1901  cagattgtta cagctgcaca gccaacacca atgactgcca ctggtgcaat
1951  gaccattgtg tccccaggaa ccacagctgc tcagaaggcc agatctccat
2001  ttttaggtat gagaattgcc ccaaggataa ccctatgtac tactgtaaca
2051  agaagaccag ctgcaggagc tgtgccctgg accagaactg ccagtgggag
2101  ccccggaatc aggagtgcat tgccctgccc gaaaatatct gtggcattgg
2151  ctggcatttg gttggaaact catgtttgaa aattactact gccaaggaga
2201  attatgacaa tgctaaattg ttctgtagga accacaatgc ccttttggct
2251  tctcttacaa cccagaagaa ggtagaattt gtccttaagc agctgcgaat
2301  aatgcagtca tctcagagca tgtccaagct caccttaacc ccatgggtcg
2351  gccttcggaa gatcaatgtg tcctactggt gctgggaaga tatgtcccca
2401  tttacaaata gtttactaca gtggatgccg tctgagccca gtgatgctgg
2451  attctgtgga attttatcag aacccagtac tcggggactg aaggctgcaa
2501  cctgcatcaa cccactcaat ggtagtgtct gtgaaaggcc tgcaaaccac
2551  agtgctaagc agtgccggac accatgtgcc ttgaggacag catgtggaga
2601  ttgcaccagc ggcagctctg agtgcatgtg gtgcagcaac atgaagcagt
```

FIGURE 10B

2651 gtgtggactc caatgcctat gtggcctcct tcccttttgg ccagtgtatg
2701 gaatggtata cgatgagcac ctgcccccct gaaaattgtt caggctactg
2751 tacctgtagt cattgcttgg agcaaccagg ctgtggctgg tgtactgatc
2801 ccagcaatac tggcaaaggg aaatgcatag agggttccta taaaggacca
2851 gtgaagatgc cttcgcaagc ccctacagga aatttctatc cacagcccct
2901 gctcaattcc agcatgtgtc tagaggacag cagatacaac tggtctttca
2951 ttcactgtcc agcttgccaa tgcaacggcc acagtaaatg catcaatcag
3001 agcatctgtg agaagtgtga gaacctgacc acaggcaagc actgcgagac
3051 ctgcatatct ggcttctacg gtgatcccac caatggaggg aaatgtcagc
3101 catgcaagtg caatgggcac gcgtctctgt gcaacaccaa cacgggcaag
3151 tgcttctgca ccaccaaggg cgtcaagggg gacgagtgcc agctatgtga
3201 ggtagaaaat cgataccaag gaaaccctct cagaggaaca tgttattata
3251 ctcttcttat tgactatcag ttcaccttta gtctatccca ggaagatgat
3301 cgctattaca cagctatcaa ttttgtggct actcctgacg aacaaaacag
3351 ggatttggac atgttcatca atgcctccaa gaatttcaac ctcaacatca
3401 cctgggctgc cagtttctca gctggaaccc aggctggaga agagatgcct
3451 gttgtttcaa aaaccaacat taaggagtac aaagatagtt tctctaatga
3501 gaagtttgat tttcgcaacc acccaaatat cactttcttt gtttatgtca
3551 gtaatttcac ctggcccatc aaaattcaga ttgccttctc tcagcacagc
3601 aattttatgg acctggtaca gttcttcgtg actttcttca gttgtttcct
3651 ctctttgctc ctggtggctg ctgtggtttg gaagatcaaa caaagttgtt
3701 gggcctccag acgtagagag caacttcttc gagagatgca acagatggcc
3751 agccgtccct ttgcctctgt aaatgtcgcc ttggaaacag atgaggagcc
3801 tcctgatctt attgggggga gtataaagac tgttcccaaa cccattgcac
3851 tggagccgtg ttttggcaac aaagccgctg tcctctctgt gtttgtgagg
3901 ctccctcgag gcctgggtgg catccctcct cctgggcagt caggtcttgc
3951 tgtggccagc gccctggtgg acatttctca gcagatgccg atagtgtaca
4001 aggagaagtc aggagccgtg agaaaccgga agcagcagcc ccctgcacag
4051 cctgggacct gcatctga

FIGURE 11

MVAAAAATEARLRRRTAATAALAGRSGGPHWDWDVTRAGRPGLGAGLRLPRLLSPPLR
PRLLLLLLLLPPPLLLLLLPCEAEAAAAAAAVSGSAAAEAKECDRPCVNGGRCNPGTG
QCVCPAGWVGEQCQHCGGRFRLTGSSGFVTDGPGNYKYKTKCTWLIEGQPNRIMRLRF
NHFATECSWDHLYVYDGDSIYAPLVAAFSGLIVPERDGNETVPEVVATSGYALLHFFS
DAAYNLTGFNITYSFDMCPNNCSGRGECKISNSSETVECECSENWKGEACDIPHCTDN
CGFPHRGICNSSDVRGCSCFSDWQGPGCSVPVPANQSFWTREEYSNLKLPRASHKAVV
NGNIMWVVGGYMFNHSDYNMVLAYDLASREWLPLNRSVNNVVVRYGHSLALYKDKIYM
YGGKIDPTGNVTNELRVFHIHNESWVLLTPKAKEQYAVVGHSAHIVTLKNGRVVMLVI
FGHCPLYGYISNVQEYDLDKNTWSILHTQGALVQGGYGHSSVYDHRTRALYVHGGYKA
FSANKYRLADDLYRYDVDTQMWTILKDSRFFRYLHTAVIVSGTMLVFGGNTHNDTSMS
HGAKCFSSDFMAYDIACDRWSVLPRPDLHHDVNRFGHSAVLHNSTMYVFGGFNSLLLS
DILVFTSEQCDAHRSEAACLAAGPGIRCVWNTGSSQCISWALATDEQEEKLKSECFSK
RTLDHDRCDQHTDCYSCTANTNDCHWCNDHCVPRNHSCSEGQISIFRYENCPKDNPMY
YCNKKTSCRSCALDQNCQWEPRNQECIALPENICGIGWHLVGNSCLKITTAKENYDNA
KLFCRNHNALLASLTTQKKVEFVLKQLRIMQSSQSMSKLTLTPWVGLRKINVSYWCWE
DMSPFTNSLLQWMPSEPSDAGFCGILSEPSTRGLKAATCINPLNGSVCERPANHSAKQ
CRTPCALRTACGDCTSGSSECMWCSNMKQCVDSNAYVASFPFGQCMEWYTMSTCPPEN
CSGYCTCSHCLEQPGCGWCTDPSNTGKGKCIEGSYKGPVKMPSQAPTGNFYPQPLLNS
SMCLEDSRYNWSFIHCPACQCNGHSKCINQSICEKCENLTTGKHCETCISGFYGDPTN
GGKCQPCKCNGHASLCNTNTGKCFCTTKGVKGDECQLCEVENRYQGNPLRGTCYYTLL
IDYQFTFSLSQEDDRYYTAINFVATPDEQNRDLDMFINASKNFNLNITWAASFSAGTQ
AGEEMPVVSKTNIKEYKDSFSNEKFDFRNHPNITFFVYVSNFTWPIKIQVQTEQ

FIGURE 12

```
1    atggtggccg cagcggcggc aactgaggca aggctgagga ggaggacggc ggcgacggca
61   gcgctcgcgg gcaggagcgg cgggccgcac tgggactggg acgtgaccag ggctgggagg
121  ccggggctgg gggccgggct gcgcctcccg cggctgctgt ctccaccgct gcggccacgg
181  ctgctgctgc tgctgttgtt gctcccgccg ccgctgttgc tgctgctgct gccctgtgag
241  gccgaggccg cggcggcggc ggcggcggtg tcgggctcag ccgcagccga ggccaaggaa
301  tgtgaccggc cctgtgtcaa cggcggtcgc tgcaaccctg gcaccggcca gtgcgtctgc
361  cccgccggct gggtgggcga gcaatgccag cactgcgggg gccgcttcag actaactgga
421  tcttctgggt ttgtgacaga tggacctgga aattataaat acaaaacgaa gtgcacgtgg
481  ctcattgaag gacagccaaa tagaataatg agacttcgtt tcaatcattt tgctacagag
541  tgtagttggg accatttata tgtttatgat ggggactcaa tttatgcacc gctagttgct
601  gcatttagtg gcctcattgt tcctgagaga gatggcaatg agactgtccc tgaggttgtt
661  gccacatcag gttatgcctt gctgcatttt tttagtgatg ctgcttataa tttgactgga
721  tttaatatta cttacagttt tgatatgtgt ccaaataact gctcaggccg aggagagtgt
781  aagatcagta atagcagcga aactgttgaa tgtgaatgtt ctgaaaactg gaaaggtgaa
841  gcatgtgaca ttcctcactg tacagacaac tgtggttttc ctcatcgagg catctgcaat
901  tcaagtgatg tcagaggatg ctcctgcttc tcagactggc agggtcctgg atgttcagtt
961  cctgtaccag ctaaccagtc attttggact cgagaggaat attctaactt aaagctcccc
1021 agagcatctc ataaagctgt ggtcaatgga aacattatgt gggttgttgg aggatatatg
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1201 aaggataaaa tttacatgta tggaggaaaa attgatccaa ctgggaatgt gaccaatgag
1261 ttgagagttt ttcacattca taatgagtca tgggtgttgt tgacccctaa ggcaaaggag
1321 cagtatgcag tggttgggca ctctgcacac attgttacac tgaagaatgg ccgagtggtc
1381 atgctggtca tctttggtca ctgccctctc tatggatata taagcaatgt gcaggaatat
1441 gatttggata agaacacatg gagtatatta cacacccagg gtgcccttgt gcaagggggt
1501 tacggccata gcagtgttta cgaccatagg accagggccc tatacgttca tggtggctac
1561 aaggctttca gtgccaataa gtaccggctt gcagatgatc tctaccgata tgatgtggat
1621 acccagatgt ggaccattct taaggacagc cgatttttcc gttacttgca cacagctgtg
1681 atagtgagtg gaaccatgct ggtgtttggg ggaaacacac acaatgacac atctatgagc
1741 catggcgcca aatgcttctc ttcagatttc atggcctatg acattgcctg tgaccgctgg
1801 tcagtgcttc ccagacctga tctccaccat gatgtcaaca gatttggcca ttcagcagtc
1861 ttacacaaca gcaccatgta tgtgttcggt ggtttcaata gtctcctcct cagcgacatc
1921 ctggtattca cctcggaaca gtgtgatgcg catcggagtg aagccgcttg tttagcagca
1981 ggacctggta ttcggtgtgt gtggaacaca gggtcgtctc agtgtatctc gtgggcgctg
2041 gcaactgatg aacaagaaga aaagttaaaa tcagaatgtt tttccaaaag aactcttgac
2101 catgacagat gtgaccagca cacagattgt tacagctgta cagccaacac caatgactgc
2161 cactggtgca atgaccattg tgtccccagg aaccacagct gctcagaagg ccagatctcc
2221 atttttaggt atgagaattg ccccaaggat aaccccatgt actactgtaa caagaagacc
2281 agctgcagga gctgtgccct ggaccagaac tgccagtggg agccccggaa tcaggagtgc
2341 attgccctgc ccgaaaatat ctgtggcatt ggctggcatt tggttggaaa ctcatgtttg
2401 aaaattacta ctgccaagga gaattatgac aatgctaaat tgttctgtag gaaccacaat
2461 gccctttttgg cttctcttac aacccagaag aaggtagaat ttgtccttaa gcagctgcga
2521 ataatgcagt catctcagag catgtccaag ctcaccttaa ccccatgggt cggccttcgg
2581 aagatcaatg tgtcctactg gtgctgggaa gatatgtccc catttacaaa tagtttacta
2641 cagtggatgc cgtctgagcc cagtgatgct ggattctgtg gaattttatc agaacccagt
2701 actcggggac tgaaggctgc aacctgcatc aacccactca atggtagtgt ctgtgaaagg
2761 cctgcaaacc acagtgctaa gcagtgccgg acaccatgtg ccttgaggac agcatgtgga
2821 gattgcacca gcggcagctc tgagtgcatg tggtgcagca acatgaagca gtgtgtggac
2881 tccaatgcct atgtggcctc cttccctttt ggccagtgta tggaatggta tacgatgagc
2941 acctgccccc ctgaaaattg ttcaggctac tgtacctgta gtcattgctt ggagcaacca
3001 ggctgtggct ggtgtactga tcccagcaat actggcaaag ggaaatgcat agagggttcc
3061 tataaaggac cagtgaagat gccttcgcaa gcccctacag gaaatttcta tccacagccc
3121 ctgctcaatt ccagcatgtg tctagaggac agcagataca actggtcttt cattcactgt
3181 ccagcttgcc aatgcaacgg ccacagtaaa tgcatcaatc agagcatctg tgagaagtgt
3241 gagaacctga ccacaggcaa gcactgcgag acctgcatat ctggcttcta cggtgatccc
3301 accaatggag ggaaatgtca gccatgcaag tgcaatgggc acgcgtctct gtgcaacacc
3361 aacacgggca agtgcttctg caccaccaag ggcgtcaagg gggacgagtg ccagctatgt
3421 gaggtagaaa atcgatacca aggaaaccct ctcagaggaa catgttatta tactcttctt
3481 attgactatc agttcacctt tagtctatcc caggaagatg atcgctatta cacagctatc
3541 aatttgtgg ctactcctga cgaacaaaac agggatttgg acatgttcat caatgcctcc
3601 aagaatttca acctcaacat cacctgggct gccagtttct cagctggaac ccaggctgga
3661 gaagagatgc ctgttgtttc aaaaaccaac attaaggagt acaaagatag tttctctaat
3721 gagaagtttg attttcgcaa ccacccaaat atcactttct ttgtttatgt cagtaatttc
3781 acctggccca tcaaaattca ggtgcaaact gaacaatga
```

FIGURE 13

1	MVAAAAATEA	RLRRRTAATA	ALAGRSGGPH	WDWDVTPAGR	PGLGAGLRLP
51	RLLSPPLRPR	LLLLLLLLPP	PLLLLLLPCE	AEAAAAAAAV	SGSAAAEAKE
101	CDRPCVNGGR	CNPGTGQCVC	PAGWVGEQCQ	HCGGRFRLTG	SSGFVTDGPG
151	NYKYKTKCTW	LIEGQPNRIM	RLRFNHFATE	CSWDHLYVYD	GDSIYAPLVA
201	AFSGLIVPER	DGNETVPEVV	ATSGYALLHF	FSDAAYNLTG	FNITYSFDMC
251	PNNCSGRGEC	KISNSSETVE	CECSENWKGE	ACDIPHCTDN	CGFPHRGICN
301	SSDVRGCSCF	SDWQGPGCSV	PVPANQSFWT	REEYSNLKLP	RASHKAVVNG
351	NIMWVVGGYM	FNHSDYNMVL	AYDLASREWL	PLNRSVNNVV	VRYGHSLALY
401	KDKIYMYGGK	IDPTGNVTNE	LRVFHIHNES	WVLLTPKAKE	QYAVVGHSAH
451	IVTLKNGRVV	MLVIFGHCPL	YGYISNVQEY	DLDKNTWSIL	HTQGALVQGG
501	YGHSSVYDHR	TRALYVHGGY	KAFSANKYRL	ADDLYRYDVD	TQMWTILKDS
551	RFFRYLHTAV	IVSGTMLVFG	GNTHNDTSMS	HGAKCFSSDF	MAYDIACDRW
601	SVLPRPDLHH	DVNRFGHSAV	LHNSTMYVFG	GFNSLLLSDI	LVFTSEQCDA
651	HRSEAACLAA	GPGIRCVWNT	GSSQCISWAL	ATDEQEEKLK	SECFSKRTLD
701	HDRCDQHTDC	YSCTANTNDC	HWCNDHCVPR	NHSCSEGQIS	IFRYENCPKD
751	NPMYYCNKKT	SCRSCALDQN	CQWEPRNQEC	IALPENICGI	GWHLVGNSCL
801	KITTAKENYD	NAKLFCRNHN	ALLASLTTQK	KVEFVLKQLR	IMQSSQSMSK
851	LTLTPWVGLR	KINVSYWCWE	DMSPFTNSLL	QWMPSEPSDA	GFCGILSEPS
901	TRGLKAATCI	NPLNGSVCER	PANHSAKQCR	TPCALRTACG	DCTSGSSECM
951	WCSNMKQCVD	SNAYVASFPF	GQCMEWYTMS	TCPPENCSGY	CTCSHCLEQP
1001	GCGWCTDPSN	TGKGKCIEGS	YKGPVKMPSQ	APTGNFYPQP	LLNSSMCLED
1051	SRYNWSFIHC	PACQCNGHSK	CINQSICEKC	ENLTTGKHCE	TCISGFYGDP
1101	TNGGKCQPCK	CNGHASLCNT	NTGKCFCTTK	GVKGDECQLC	EVENRYQGNP
1151	LRGTCYYTLL	IDYQFTFSLS	QEDDRYYTAI	NFVATPDEQN	RDLDMFINAS
1201	KNFNLNITWA	ASFSAGTQAG	EEMPVVSKTN	IKEYKDSFSN	EKFDFRNHPN
1251	ITFFVYVSNF	TWPIKIQIAF	SQHSNFMDLV	QFFVTFFSCF	LSLLLVAAVV
1301	WKIKQSCWAS	RRREQLLREM	QQMASRPFAS	VNVALETDEE	PPDLIGGSIK
1351	TVPKPIALEP	CFGNKAAVLS	VFVRLPRGLG	GIPPPGQSGL	AVASALVDIS
1401	QQMPIVYKEK	SGAVRNRKQQ	PPAQPGTCI		

FIGURE 14A

```
   1 atggtggccg cagcggcggc aactgaggca aggctgagga ggaggacggc
  51 ggcgacggca gcgctcgcgg gcaggagcgg cgggccgcac tgggactggg
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 151 cggctgctgt ctccaccgct gcggccacgg ctgctgctgc tgctgttgtt
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 251 cggcggcggc ggcggcggtg tcgggctcag ccgcagccga ggccaaggaa
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 351 gtgcgtctgc cccgccggct gggtgggcga gcaatgccag cactgcgggg
 401 gccgcttcag actaactgga tcttctgggt ttgtgacaga tggacctgga
 451 aattataaat acaaaacgaa gtgcacgtgg ctcattgaag gacagccaaa
 501 tagaataatg agacttcgtt tcaatcattt tgctacagag tgtagttggg
 551 accatttata tgtttatgat ggggactcaa tttatgcacc gctagttgct
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1401 ctgccctctc tatggatata taagcaatgt gcaggaatat gatttggata
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1501 tacggccata gcagtgttta cgaccatagg accagggccc tatacgttca
1551 tggtggctac aaggctttca gtgccaataa gtaccggctt gcagatgatc
1601 tctaccgata tgatgtggat acccagatgt ggaccattct taaggacagc
1651 cgatttttcc gttacttgca cacagctgtg atagtgagtg gaaccatgct
1701 ggtgtttggg ggaaacacac acaatgacac atctatgagc catggcgcca
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2151 caatgactgc cactggtgca atgaccattg tgtccccagg aaccacagct
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2251 aaccccatgt actactgtaa caagaagacc agctgcagga gctgtgccct
2301 ggaccagaac tgccagtggg agccccggaa tcaggagtgc attgccctgc
2351 ccgaaaatat ctgtggcatt ggctggcatt tggttggaaa ctcatgtttg
2401 aaaattacta ctgccaagga gaattatgac aatgctaaat tgttctgtag
2451 gaaccacaat gccctttttgg cttctcttac aacccagaag aaggtagaat
2501 ttgtccttaa gcagctgcga ataatgcagt catctcagag catgtccaag
2551 ctcaccttaa ccccatgggt cggccttcgg aagatcaatg tgtcctactg
2601 gtgctgggaa gatatgtccc catttacaaa tagtttacta cagtggatgc
```

Figure 14B

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2651  cgtctgagcc cagtgatgct ggattctgtg gaattttatc agaacccagt
2701  actcggggac tgaaggctgc aacctgcatc aacccactca atggtagtgt
2751  ctgtgaaagg cctgcaaacc acagtgctaa gcagtgccgg acaccatgtg
2801  ccttgaggac agcatgtgga gattgcacca gcggcagctc tgagtgcatg
2851  tggtgcagca acatgaagca gtgtgtggac tccaatgcct atgtggcctc
2901  cttccctttt ggccagtgta tggaatggta tacgatgagc acctgccccc
2951  ctgaaaattg ttcaggctac tgtacctgta gtcattgctt ggagcaacca
3001  ggctgtggct ggtgtactga tcccagcaat actggcaaag ggaaatgcat
3051  agagggttcc tataaaggac cagtgaagat gccttcgcaa gcccctacag
3101  gaaatttcta tccacagccc ctgctcaatt ccagcatgtg tctagaggac
3151  agcagataca actggtcttt cattcactgt ccagcttgcc aatgcaacgg
3201  ccacagtaaa tgcatcaatc agagcatctg tgagaagtgt gagaacctga
3251  ccacaggcaa gcactgcgag acctgcatat ctggcttcta cggtgatccc
3301  accaatggag ggaaatgtca gccatgcaag tgcaatgggc acgcgtctct
3351  gtgcaacacc aacacgggca agtgcttctg caccaccaag ggcgtcaagg
3401  gggacgagtg ccagctatgt gaggtagaaa atcgatacca aggaaaccct
3451  ctcagaggaa catgttatta tactcttctt attgactatc agttcacctt
3501  tagtctatcc caggaagatg atcgctatta cacagctatc aattttgtgg
3551  ctactcctga cgaacaaaac agggatttgg acatgttcat caatgcctcc
3601  aagaatttca acctcaacat cacctgggct gccagtttct cagctggaac
3651  ccaggctgga gaagagatgc ctgttgtttc aaaaaccaac attaaggagt
3701  acaaagatag tttctctaat gagaagtttg attttcgcaa ccacccaaat
3751  atcactttct ttgtttatgt cagtaatttc acctggccca tcaaaattca
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4101  tgtcctctct gtgtttgtga ggctccctcg aggcctgggt ggcatccctc
4151  ctcctgggca gtcaggtctt gctgtggcca gcgccctggt ggacatttct
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4251  gaagcagcag ccccctgcac agcctgggac ctgcatctga
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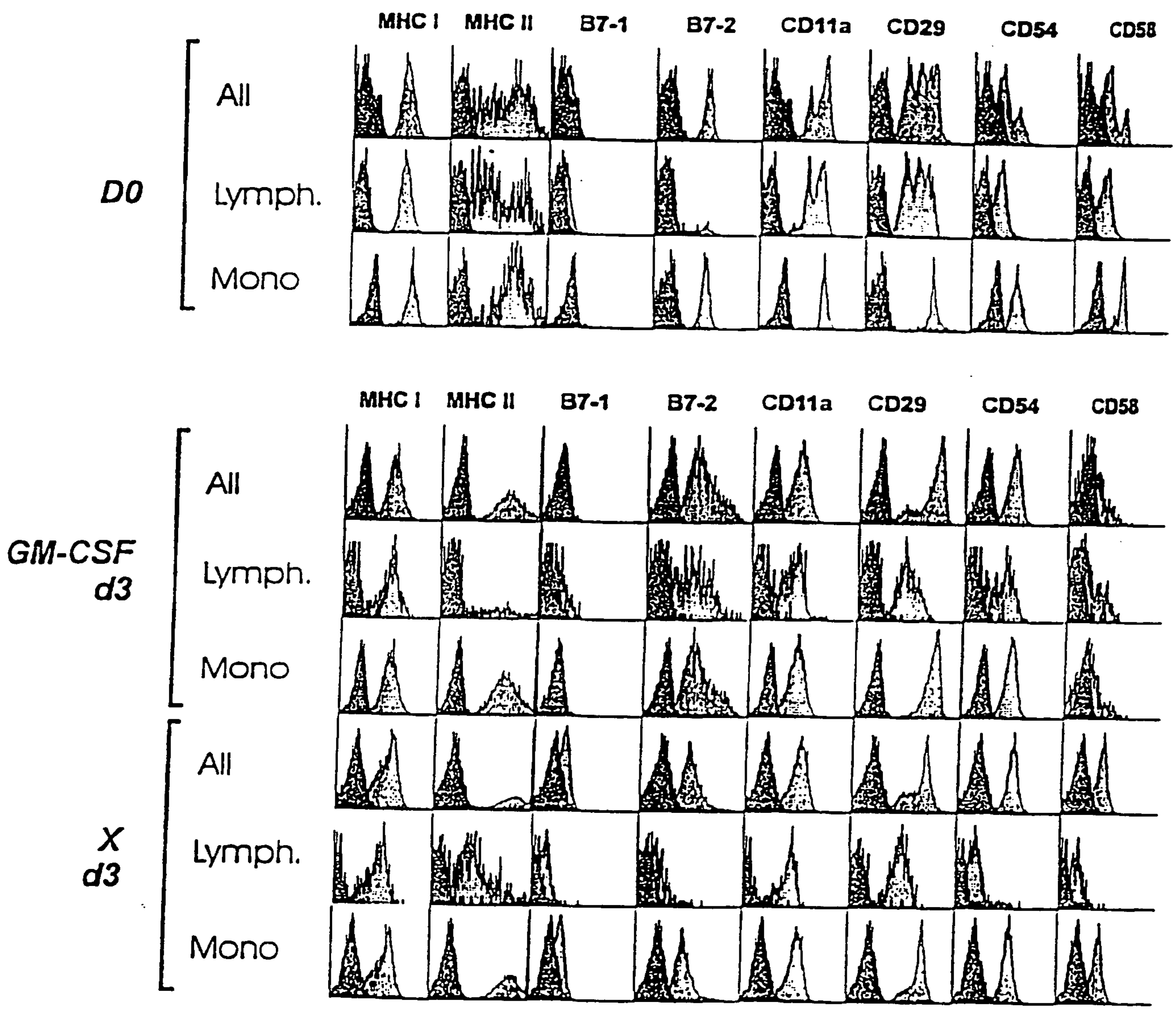
MHC I
MHC II
B7-1
B7-2
CD11a
CD29
CD54
CD58
D0
All
Lymph.
Mono
GM-CSF
d3
X
d3

Fig. 15

SEQUENCE LISTING

<110> Dana-Farber Cancer Institute, Inc.

<120> REGULATION OF IMMUNE RESPONSES BY ATTRACTIN

<130> 00530/089WO1

<150> US 60/100,137
<151> 1998-09-14

<160> 25

<170> FastSEQ for Windows Version 3.0

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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(3594)

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Met Val Ala Ala Ala Ala Ala Thr Glu Ala Arg Leu Arg Arg Arg Thr
1 5 10 15

gcg gcg acg gca gcg ctc gcg ggc agg agc ggc ggg ccg cac tgt gtc 96
Ala Ala Thr Ala Ala Leu Ala Gly Arg Ser Gly Gly Pro His Cys Val
20 25 30

aac ggc ggt cgc tgc aac cct ggc acc ggc cag tgc gtc tgc ccc gcc 144
Asn Gly Gly Arg Cys Asn Pro Gly Thr Gly Gln Cys Val Cys Pro Ala
35 40 45

ggc tgg gtg ggc gag caa tgc cag cac tgc ggg ggc cgc ttc aga cta 192
Gly Trp Val Gly Glu Gln Cys Gln His Cys Gly Gly Arg Phe Arg Leu
50 55 60

act gga tct tct ggg ttt gtg aca gat gga cct gga aat tat aaa tac 240
Thr Gly Ser Ser Gly Phe Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr
65 70 75 80

aaa acg aag tgc acg tgg ctc att gaa gga cag cca aat aga ata atg 288
Lys Thr Lys Cys Thr Trp Leu Ile Glu Gly Gln Pro Asn Arg Ile Met
85 90 95

aga ctt cgt ttc aat cat ttt gct aca gag tgt agt tgg gac cat tta 336
Arg Leu Arg Phe Asn His Phe Ala Thr Glu Cys Ser Trp Asp His Leu
100 105 110

tat gtt tat gat ggg gac tca att tat gca ccg cta gtt gct gca ttt 384
Tyr Val Tyr Asp Gly Asp Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe
115 120 125

agt ggc ctc att gtt cct gag aga gat ggc aat gag act gtc cct gag 432
Ser Gly Leu Ile Val Pro Glu Arg Asp Gly Asn Glu Thr Val Pro Glu
130 135 140

gtt gtt gcc aca tca ggt tat gcc ttg ctg cat ttt ttt agt gat gct 480
Val Val Ala Thr Ser Gly Tyr Ala Leu Leu His Phe Phe Ser Asp Ala
145 150 155 160

gct tat aat ttg act gga ttt aat att act tac agt ttt gat atg tgt 528
Ala Tyr Asn Leu Thr Gly Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys
165 170 175

cca aat aac tgc tca ggc cga gga gag tgt aag atc agt aat agc agc 576
Pro Asn Asn Cys Ser Gly Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser
180 185 190

gat act gtt gaa tgt gaa tgt tct gaa aac tgg aaa ggt gaa gca tgt 624
Asp Thr Val Glu Cys Glu Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys
195 200 205

gac att cct cac tgt aca gac aac tgt ggt ttt cct cat cga ggc atc 672
Asp Ile Pro His Cys Thr Asp Asn Cys Gly Phe Pro His Arg Gly Ile
210 215 220

tgc aat tca agt gat gtc aga gga tgc tcc tgc ttc tca gac tgg cag 720
Cys Asn Ser Ser Asp Val Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln
225 230 235 240

ggt cct gga tgt tca gtt cct gta cca gct aac cag tca ttt tgg act 768
Gly Pro Gly Cys Ser Val Pro Val Pro Ala Asn Gln Ser Phe Trp Thr
245 250 255

cga gag gaa tat tct aac tta aag ctc ccc aga gca tct cat aaa gct 816
Arg Glu Glu Tyr Ser Asn Leu Lys Leu Pro Arg Ala Ser His Lys Ala
260 265 270

gtg gtc aat gga aac att atg tgg gtt gtt gga gga tat atg ttc aac 864
Val Val Asn Gly Asn Ile Met Trp Val Val Gly Gly Tyr Met Phe Asn
275 280 285

cac tca gat tat aac atg gtt cta gcg tat gac ctt gct tct agg gag 912
His Ser Asp Tyr Asn Met Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu
290 295 300

tgg ctt cca cta aac cgt tct gtg aac aat gtg gtt gtt aga tat ggt 960
Trp Leu Pro Leu Asn Arg Ser Val Asn Asn Val Val Val Arg Tyr Gly
305 310 315 320

cat tct ttg gca tta tac aag gat aaa att tac atg tat gga gga aaa 1008
His Ser Leu Ala Leu Tyr Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys
325 330 335

att gat tca act ggg aat gtg acc aat gag ttg aga gtt ttt cac att 1056
Ile Asp Ser Thr Gly Asn Val Thr Asn Glu Leu Arg Val Phe His Ile
340 345 350

cat aat gag tca tgg gtg ttg ttg acc cct aag gca aag gag cag tat 1104
His Asn Glu Ser Trp Val Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr
355 360 365

gca gtg gtt ggg cac tct gca cac att gtt aca ctg aag aat ggc cga 1152
Ala Val Val Gly His Ser Ala His Ile Val Thr Leu Lys Asn Gly Arg
370 375 380

gtg gtc atg ctg gtc atc ttt ggt cac tgc cct ctc tat gga tat ata 1200
Val Val Met Leu Val Ile Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile
385 390 395 400

agc aat gtg cag gaa tat gat ttg gat aag aac aca tgg agt ata tta 1248
Ser Asn Val Gln Glu Tyr Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu
405 410 415

cac acc cag ggt gcc ctt gtg caa ggg ggt tac ggc cat agc agt gtt 1296
His Thr Gln Gly Ala Leu Val Gln Gly Gly Tyr Gly His Ser Ser Val
420 425 430

tac gac cat agg acc agg gcc cta tac gtt cat ggt ggc tac aag gct 1344
Tyr Asp His Arg Thr Arg Ala Leu Tyr Val His Gly Gly Tyr Lys Ala
435 440 445

ttc agt gcc aat aag tac cgg ctt gca gat gat ctc tac cga tat gat 1392
Phe Ser Ala Asn Lys Tyr Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp
450 455 460

gtg gat acc cag atg tgg acc att ctt aag gac agc cga ttt ttc cgt 1440
Val Asp Thr Gln Met Trp Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg
465 470 475 480

tac ttg cac aca gct gtg ata gtg agt gga acc atg ctg gtg ttt ggg 1488
Tyr Leu His Thr Ala Val Ile Val Ser Gly Thr Met Leu Val Phe Gly
485 490 495

gga aac aca cac aat gac aca tct atg agc cat ggc gcc aaa tgc ttc 1536
Gly Asn Thr His Asn Asp Thr Ser Met Ser His Gly Ala Lys Cys Phe
500 505 510

tct tca gat ttc atg gcc tat gac att gcc tgt gac cgc tgg tca gtg 1584
Ser Ser Asp Phe Met Ala Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val
515 520 525

ctt ccc aga cct gat tcc acc atg atg tca aca gat ttg gcc att cca 1632
Leu Pro Arg Pro Asp Ser Thr Met Met Ser Thr Asp Leu Ala Ile Pro
530 535 540

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Ala Val Leu His Asn Ser Thr Met Tyr Val Phe Gly Gly Phe Asn Ser
545 550 555 560

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Leu Leu Leu Ser Asp Ile Leu Val Phe Thr Ser Glu Gln Cys Asp Ala
565 570 575

cat cgg agt gaa gcc gct tgt tta gca gca gga cct ggt att cgg tgt 1776
His Arg Ser Glu Ala Ala Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys
580 585 590

gtg tgg aac aca ggg tcg tct cag tgt atc tcg tgg gcg ctg gca act 1824
Val Trp Asn Thr Gly Ser Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr
595 600 605

gat gaa caa gaa gaa aag tta aaa tca gaa tgt ttt tcc aaa aga act 1872
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610 615 620

ctt gac cat gac aga tgt gac cag cac aca gat tgt tac agc tgc aca 1920
Leu Asp His Asp Arg Cys Asp Gln His Thr Asp Cys Tyr Ser Cys Thr
625 630 635 640

gcc aac acc aat gac tgc cac tgg tgc aat gac cat tgt gtc ccc agg 1968
Ala Asn Thr Asn Asp Cys His Trp Cys Asn Asp His Cys Val Pro Arg
645 650 655

aac cac agc tgc tca gaa ggc cag atc tcc att ttt agg tat gag aat 2016
Asn His Ser Cys Ser Glu Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn
660 665 670

tgc ccc aag gat aac cct atg tac tac tgt aac aag aag acc agc tgc 2064
Cys Pro Lys Asp Asn Pro Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys
675 680 685

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Arg Ser Cys Ala Leu Asp Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln
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gag tgc att gcc ctg ccc gaa aat atc tgt ggc att ggc tgg cat ttg     2160
Glu Cys Ile Ala Leu Pro Glu Asn Ile Cys Gly Ile Gly Trp His Leu
705                 710                 715                 720

gtt gga aac tca tgt ttg aaa att act act gcc aag gag aat tat gac     2208
Val Gly Asn Ser Cys Leu Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp
                725                 730                 735

aat gct aaa ttg ttc tgt agg aac cac aat gcc ctt ttg gct tct ctt     2256
Asn Ala Lys Leu Phe Cys Arg Asn His Asn Ala Leu Leu Ala Ser Leu
            740                 745                 750

aca acc cag aag aag gta gaa ttt gtc ctt aag cag ctg cga ata atg     2304
Thr Thr Gln Lys Lys Val Glu Phe Val Leu Lys Gln Leu Arg Ile Met
        755                 760                 765

cag tca tct cag agc atg tcc aag ctc acc tta acc cca tgg gtc ggc     2352
Gln Ser Ser Gln Ser Met Ser Lys Leu Thr Leu Thr Pro Trp Val Gly
    770                 775                 780

ctt cgg aag atc aat gtg tcc tac tgg tgc tgg gaa gat atg tcc cca     2400
Leu Arg Lys Ile Asn Val Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro
785                 790                 795                 800

ttt aca aat agt tta cta cag tgg atg ccg tct gag ccc agt gat gct     2448
Phe Thr Asn Ser Leu Leu Gln Trp Met Pro Ser Glu Pro Ser Asp Ala
                805                 810                 815

gga ttc tgt gga att tta tca gaa ccc agt act cgg gga ctg aag gct     2496
Gly Phe Cys Gly Ile Leu Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala
            820                 825                 830

gca acc tgc atc aac cca ctc aat ggt agt gtc tgt gaa agg cct gca     2544
Ala Thr Cys Ile Asn Pro Leu Asn Gly Ser Val Cys Glu Arg Pro Ala
        835                 840                 845

aac cac agt gct aag cag tgc cgg aca cca tgt gcc ttg agg aca gca     2592
Asn His Ser Ala Lys Gln Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala
    850                 855                 860

tgt gga gat tgc acc agc ggc agc tct gag tgc atg tgg tgc agc aac     2640
Cys Gly Asp Cys Thr Ser Gly Ser Ser Glu Cys Met Trp Cys Ser Asn
865                 870                 875                 880

atg aag cag tgt gtg gac tcc aat gcc tat gtg gcc tcc ttc cct ttt     2688
Met Lys Gln Cys Val Asp Ser Asn Ala Tyr Val Ala Ser Phe Pro Phe
                885                 890                 895

ggc cag tgt atg gaa tgg tat acg atg agc acc tgc ccc cct gaa aat     2736
Gly Gln Cys Met Glu Trp Tyr Thr Met Ser Thr Cys Pro Pro Glu Asn
            900                 905                 910

tgt tca ggc tac tgt acc tgt agt cat tgc ttg gag caa cca ggc tgt     2784
Cys Ser Gly Tyr Cys Thr Cys Ser His Cys Leu Glu Gln Pro Gly Cys
        915                 920                 925

ggc tgg tgt act gat ccc agc aat act ggc aaa ggg aaa tgc ata gag     2832
Gly Trp Cys Thr Asp Pro Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu
    930                 935                 940

ggt tcc tat aaa gga cca gtg aag atg cct tcg caa gcc cct aca gga     2880
Gly Ser Tyr Lys Gly Pro Val Lys Met Pro Ser Gln Ala Pro Thr Gly
945                 950                 955                 960
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aat ttc tat cca cag ccc ctg ctc aat tcc agc atg tgt cta gag gac 2928
Asn Phe Tyr Pro Gln Pro Leu Leu Asn Ser Ser Met Cys Leu Glu Asp
965 970 975

agc aga tac aac tgg tct ttc att cac tgt cca gct tgc caa tgc aac 2976
Ser Arg Tyr Asn Trp Ser Phe Ile His Cys Pro Ala Cys Gln Cys Asn
980 985 990

ggc cac agt aaa tgc atc aat cag agc atc tgt gag aag tgt gag aac 3024
Gly His Ser Lys Cys Ile Asn Gln Ser Ile Cys Glu Lys Cys Glu Asn
995 1000 1005

ctg acc aca ggc aag cac tgc gag acc tgc ata tct ggc ttc tac ggt 3072
Leu Thr Thr Gly Lys His Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly
1010 1015 1020

gat ccc acc aat gga ggg aaa tgt cag cca tgc aag tgc aat ggg cac 3120
Asp Pro Thr Asn Gly Gly Lys Cys Gln Pro Cys Lys Cys Asn Gly His
1025 1030 1035 1040

gcg tct ctg tgc aac acc aac acg ggc aag tgc ttc tgc acc acc aag 3168
Ala Ser Leu Cys Asn Thr Asn Thr Gly Lys Cys Phe Cys Thr Thr Lys
1045 1050 1055

ggc gtc aag ggg gac gag tgc cag cta tgt gag gta gaa aat cga tac 3216
Gly Val Lys Gly Asp Glu Cys Gln Leu Cys Glu Val Glu Asn Arg Tyr
1060 1065 1070

caa gga aac cct ctc aga gga aca tgt tat tat act ctt ctt att gac 3264
Gln Gly Asn Pro Leu Arg Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp
1075 1080 1085

tat cag ttc acc ttt agt cta tcc cag gaa gat gat cgc tat tac aca 3312
Tyr Gln Phe Thr Phe Ser Leu Ser Gln Glu Asp Asp Arg Tyr Tyr Thr
1090 1095 1100

gct atc aat ttt gtg gct act cct gac gaa caa aac agg gat ttg gac 3360
Ala Ile Asn Phe Val Ala Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp
1105 1110 1115 1120

atg ttc atc aat gcc tcc aag aat ttc aac ctc aac atc acc tgg gct 3408
Met Phe Ile Asn Ala Ser Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala
1125 1130 1135

gcc agt ttc tca gct gga acc cag gct gga gaa gag atg cct gtt gtt 3456
Ala Ser Phe Ser Ala Gly Thr Gln Ala Gly Glu Glu Met Pro Val Val
1140 1145 1150

tca aaa acc aac att aag gag tac aaa gat agt ttc tct aat gag aag 3504
Ser Lys Thr Asn Ile Lys Glu Tyr Lys Asp Ser Phe Ser Asn Glu Lys
1155 1160 1165

ttt gat ttt cgc aac cac cca aat atc act ttc ttt gtt tat gtc agt 3552
Phe Asp Phe Arg Asn His Pro Asn Ile Thr Phe Phe Val Tyr Val Ser
1170 1175 1180

aat ttc acc tgg ccc atc aaa att cag gtg caa act gaa caa 3594
Asn Phe Thr Trp Pro Ile Lys Ile Gln Val Gln Thr Glu Gln
1185 1190 1195

tga 3597

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<212> PRT
<213> Homo sapiens

<400> 2

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Asn Gly Gly Arg Cys Asn Pro Gly Thr Gly Gln Cys Val Cys Pro Ala
35 40 45
Gly Trp Val Gly Glu Gln Cys Gln His Cys Gly Gly Arg Phe Arg Leu
50 55 60
Thr Gly Ser Ser Gly Phe Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr
65 70 75 80
Lys Thr Lys Cys Thr Trp Leu Ile Glu Gly Gln Pro Asn Arg Ile Met
85 90 95
Arg Leu Arg Phe Asn His Phe Ala Thr Glu Cys Ser Trp Asp His Leu
100 105 110
Tyr Val Tyr Asp Gly Asp Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe
115 120 125
Ser Gly Leu Ile Val Pro Glu Arg Asp Gly Asn Glu Thr Val Pro Glu
130 135 140
Val Val Ala Thr Ser Gly Tyr Ala Leu Leu His Phe Phe Ser Asp Ala
145 150 155 160
Ala Tyr Asn Leu Thr Gly Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys
165 170 175
Pro Asn Asn Cys Ser Gly Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser
180 185 190
Asp Thr Val Glu Cys Glu Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys
195 200 205
Asp Ile Pro His Cys Thr Asp Asn Cys Gly Phe Pro His Arg Gly Ile
210 215 220
Cys Asn Ser Ser Asp Val Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln
225 230 235 240
Gly Pro Gly Cys Ser Val Pro Val Pro Ala Asn Gln Ser Phe Trp Thr
245 250 255
Arg Glu Glu Tyr Ser Asn Leu Lys Leu Pro Arg Ala Ser His Lys Ala
260 265 270
Val Val Asn Gly Asn Ile Met Trp Val Val Gly Gly Tyr Met Phe Asn
275 280 285
His Ser Asp Tyr Asn Met Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu
290 295 300
Trp Leu Pro Leu Asn Arg Ser Val Asn Asn Val Val Val Arg Tyr Gly
305 310 315 320
His Ser Leu Ala Leu Tyr Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys
325 330 335
Ile Asp Ser Thr Gly Asn Val Thr Asn Glu Leu Arg Val Phe His Ile
340 345 350
His Asn Glu Ser Trp Val Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr
355 360 365
Ala Val Val Gly His Ser Ala His Ile Val Thr Leu Lys Asn Gly Arg
370 375 380
Val Val Met Leu Val Ile Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile
385 390 395 400
Ser Asn Val Gln Glu Tyr Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu
405 410 415
His Thr Gln Gly Ala Leu Val Gln Gly Gly Tyr Gly His Ser Ser Val
420 425 430
Tyr Asp His Arg Thr Arg Ala Leu Tyr Val His Gly Gly Tyr Lys Ala
435 440 445
Phe Ser Ala Asn Lys Tyr Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp
450 455 460
Val Asp Thr Gln Met Trp Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg
465 470 475 480
Tyr Leu His Thr Ala Val Ile Val Ser Gly Thr Met Leu Val Phe Gly
485 490 495
Gly Asn Thr His Asn Asp Thr Ser Met Ser His Gly Ala Lys Cys Phe
500 505 510
Ser Ser Asp Phe Met Ala Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val
515 520 525

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Leu Pro Arg Pro Asp Ser Thr Met Met Ser Thr Asp Leu Ala Ile Pro
    530                 535                 540
Ala Val Leu His Asn Ser Thr Met Tyr Val Phe Gly Gly Phe Asn Ser
545                 550                 555                 560
Leu Leu Leu Ser Asp Ile Leu Val Phe Thr Ser Glu Gln Cys Asp Ala
                565                 570                 575
His Arg Ser Glu Ala Ala Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys
            580                 585                 590
Val Trp Asn Thr Gly Ser Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr
        595                 600                 605
Asp Glu Gln Glu Glu Lys Leu Lys Ser Glu Cys Phe Ser Lys Arg Thr
    610                 615                 620
Leu Asp His Asp Arg Cys Asp Gln His Thr Asp Cys Tyr Ser Cys Thr
625                 630                 635                 640
Ala Asn Thr Asn Asp Cys His Trp Cys Asn Asp His Cys Val Pro Arg
                645                 650                 655
Asn His Ser Cys Ser Glu Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn
            660                 665                 670
Cys Pro Lys Asp Asn Pro Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys
        675                 680                 685
Arg Ser Cys Ala Leu Asp Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln
    690                 695                 700
Glu Cys Ile Ala Leu Pro Glu Asn Ile Cys Gly Ile Gly Trp His Leu
705                 710                 715                 720
Val Gly Asn Ser Cys Leu Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp
                725                 730                 735
Asn Ala Lys Leu Phe Cys Arg Asn His Asn Ala Leu Leu Ala Ser Leu
            740                 745                 750
Thr Thr Gln Lys Lys Val Glu Phe Val Leu Lys Gln Leu Arg Ile Met
        755                 760                 765
Gln Ser Ser Gln Ser Met Ser Lys Leu Thr Leu Thr Pro Trp Val Gly
    770                 775                 780
Leu Arg Lys Ile Asn Val Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro
785                 790                 795                 800
Phe Thr Asn Ser Leu Leu Gln Trp Met Pro Ser Glu Pro Ser Asp Ala
                805                 810                 815
Gly Phe Cys Gly Ile Leu Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala
            820                 825                 830
Ala Thr Cys Ile Asn Pro Leu Asn Gly Ser Val Cys Glu Arg Pro Ala
        835                 840                 845
Asn His Ser Ala Lys Gln Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala
    850                 855                 860
Cys Gly Asp Cys Thr Ser Gly Ser Ser Glu Cys Met Trp Cys Ser Asn
865                 870                 875                 880
Met Lys Gln Cys Val Asp Ser Asn Ala Tyr Val Ala Ser Phe Pro Phe
                885                 890                 895
Gly Gln Cys Met Glu Trp Tyr Thr Met Ser Thr Cys Pro Pro Glu Asn
            900                 905                 910
Cys Ser Gly Tyr Cys Thr Cys Ser His Cys Leu Glu Gln Pro Gly Cys
        915                 920                 925
Gly Trp Cys Thr Asp Pro Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu
    930                 935                 940
Gly Ser Tyr Lys Gly Pro Val Lys Met Pro Ser Gln Ala Pro Thr Gly
945                 950                 955                 960
Asn Phe Tyr Pro Gln Pro Leu Leu Asn Ser Ser Met Cys Leu Glu Asp
                965                 970                 975
Ser Arg Tyr Asn Trp Ser Phe Ile His Cys Pro Ala Cys Gln Cys Asn
            980                 985                 990
Gly His Ser Lys Cys Ile Asn Gln Ser Ile Cys Glu Lys Cys Glu Asn
        995                 1000                1005
Leu Thr Thr Gly Lys His Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly
    1010                1015                1020
Asp Pro Thr Asn Gly Gly Lys Cys Gln Pro Cys Lys Cys Asn Gly His
1025                1030                1035                1040
Ala Ser Leu Cys Asn Thr Asn Thr Gly Lys Cys Phe Cys Thr Thr Lys
                1045                1050                1055
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Gly Val Lys Gly Asp Glu Cys Gln Leu Cys Glu Val Glu Asn Arg Tyr
1060 1065 1070
Gln Gly Asn Pro Leu Arg Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp
1075 1080 1085
Tyr Gln Phe Thr Phe Ser Leu Ser Gln Glu Asp Asp Arg Tyr Tyr Thr
1090 1095 1100
Ala Ile Asn Phe Val Ala Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp
1105 1110 1115 1120
Met Phe Ile Asn Ala Ser Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala
1125 1130 1135
Ala Ser Phe Ser Ala Gly Thr Gln Ala Gly Glu Glu Met Pro Val Val
1140 1145 1150
Ser Lys Thr Asn Ile Lys Glu Tyr Lys Asp Ser Phe Ser Asn Glu Lys
1155 1160 1165
Phe Asp Phe Arg Asn His Pro Asn Ile Thr Phe Phe Val Tyr Val Ser
1170 1175 1180
Asn Phe Thr Trp Pro Ile Lys Ile Gln Val Gln Thr Glu Gln
1185 1190 1195

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<213> Homo sapiens

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<211> 32
<212> DNA
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cccaagcttg ggatggtggc cgcagcggcg gc 32

<210> 6
<211> 25
<212> DNA
<213> Homo sapiens

<400> 6
ccaggtccat ctgtcacaaa cccag 25

<210> 7
<211> 38
<212> DNA
<213> Homo sapiens

<400> 7
gtgcgtgaag cttgtaccgg caactgaggc aaggctga 38

<210> 8
<211> 39
<212> DNA
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<400> 8
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<213> Artificial Sequence

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<210> 10
<211> 1355
<212> PRT
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Ala Ala Thr Ala Ala Leu Ala Gly Arg Ser Gly Gly Pro His Cys Val
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Asn Gly Gly Arg Cys Asn Pro Gly Thr Gly Gln Cys Val Cys Pro Ala
35 40 45
Gly Trp Val Gly Glu Gln Cys Gln His Cys Gly Gly Arg Phe Arg Leu
50 55 60
Thr Gly Ser Ser Gly Phe Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr
65 70 75 80
Lys Thr Lys Cys Thr Trp Leu Ile Glu Gly Gln Pro Asn Arg Ile Met
85 90 95
Arg Leu Arg Phe Asn His Phe Ala Thr Glu Cys Ser Trp Asp His Leu
100 105 110
Tyr Val Tyr Asp Gly Asp Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe
115 120 125
Ser Gly Leu Ile Val Pro Glu Arg Asp Gly Asn Glu Thr Val Pro Glu
130 135 140
Val Val Ala Thr Ser Gly Tyr Ala Leu Leu His Phe Phe Ser Asp Ala
145 150 155 160
Ala Tyr Asn Leu Thr Gly Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys
165 170 175
Pro Asn Asn Cys Ser Gly Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser
180 185 190
Asp Thr Val Glu Cys Glu Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys
195 200 205
Asp Ile Pro His Cys Thr Asp Asn Cys Gly Phe Pro His Arg Gly Ile
210 215 220
Cys Asn Ser Ser Asp Val Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln
225 230 235 240
Gly Pro Gly Cys Ser Val Pro Val Pro Ala Asn Gln Ser Phe Trp Thr
245 250 255
Arg Glu Glu Tyr Ser Asn Leu Lys Leu Pro Arg Ala Ser His Lys Ala
260 265 270
Val Val Asn Gly Asn Ile Met Trp Val Val Gly Gly Tyr Met Phe Asn
275 280 285
His Ser Asp Tyr Asn Met Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu
290 295 300
Trp Leu Pro Leu Asn Arg Ser Val Asn Asn Val Val Val Arg Tyr Gly
305 310 315 320
His Ser Leu Ala Leu Tyr Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys
325 330 335
Ile Asp Ser Thr Gly Asn Val Thr Asn Glu Leu Arg Val Phe His Ile
340 345 350
His Asn Glu Ser Trp Val Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr
355 360 365
Ala Val Val Gly His Ser Ala His Ile Val Thr Leu Lys Asn Gly Arg
370 375 380

Val Val Met Leu Val Ile Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile
385 390 395 400
Ser Asn Val Gln Glu Tyr Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu
405 410 415
His Thr Gln Gly Ala Leu Val Gln Gly Gly Tyr Gly His Ser Ser Val
420 425 430
Tyr Asp His Arg Thr Arg Ala Leu Tyr Val His Gly Gly Tyr Lys Ala
435 440 445
Phe Ser Ala Asn Lys Tyr Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp
450 455 460
Val Asp Thr Gln Met Trp Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg
465 470 475 480
Tyr Leu His Thr Ala Val Ile Val Ser Gly Thr Met Leu Val Phe Gly
485 490 495
Gly Asn Thr His Asn Asp Thr Ser Met Ser His Gly Ala Lys Cys Phe
500 505 510
Ser Ser Asp Phe Met Ala Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val
515 520 525
Leu Pro Arg Pro Asp Leu His His Asp Val Asn Arg Phe Gly His Ser
530 535 540
Ala Val Leu His Asn Ser Thr Met Tyr Val Phe Gly Gly Phe Asn Ser
545 550 555 560
Leu Leu Leu Ser Asp Ile Leu Val Phe Thr Ser Glu Gln Cys Asp Ala
565 570 575
His Arg Ser Glu Ala Ala Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys
580 585 590
Val Trp Asn Thr Gly Ser Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr
595 600 605
Asp Glu Gln Glu Glu Lys Leu Lys Ser Glu Cys Phe Ser Lys Arg Thr
610 615 620
Leu Asp His Asp Arg Cys Asp Gln His Thr Asp Cys Tyr Ser Cys Thr
625 630 635 640
Ala Asn Thr Asn Asp Cys His Trp Cys Asn Asp His Cys Val Pro Arg
645 650 655
Asn His Ser Cys Ser Glu Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn
660 665 670
Cys Pro Lys Asp Asn Pro Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys
675 680 685
Arg Ser Cys Ala Leu Asp Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln
690 695 700
Glu Cys Ile Ala Leu Pro Glu Asn Ile Cys Gly Ile Gly Trp His Leu
705 710 715 720
Val Gly Asn Ser Cys Leu Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp
725 730 735
Asn Ala Lys Leu Phe Cys Arg Asn His Asn Ala Leu Leu Ala Ser Leu
740 745 750
Thr Thr Gln Lys Lys Val Glu Phe Val Leu Lys Gln Leu Arg Ile Met
755 760 765
Gln Ser Ser Gln Ser Met Ser Lys Leu Thr Leu Thr Pro Trp Val Gly
770 775 780
Leu Arg Lys Ile Asn Val Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro
785 790 795 800
Phe Thr Asn Ser Leu Leu Gln Trp Met Pro Ser Glu Pro Ser Asp Ala
805 810 815
Gly Phe Cys Gly Ile Leu Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala
820 825 830
Ala Thr Cys Ile Asn Pro Leu Asn Gly Ser Val Cys Glu Arg Pro Ala
835 840 845
Asn His Ser Ala Lys Gln Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala
850 855 860
Cys Gly Asp Cys Thr Ser Gly Ser Ser Glu Cys Met Trp Cys Ser Asn
865 870 875 880
Met Lys Gln Cys Val Asp Ser Asn Ala Tyr Val Ala Ser Phe Pro Phe
885 890 895
Gly Gln Cys Met Glu Trp Tyr Thr Met Ser Thr Cys Pro Pro Glu Asn
900 905 910

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Cys Ser Gly Tyr Cys Thr Cys Ser His Cys Leu Glu Gln Pro Gly Cys
        915                 920                 925
Gly Trp Cys Thr Asp Pro Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu
    930                 935                 940
Gly Ser Tyr Lys Gly Pro Val Lys Met Pro Ser Gln Ala Pro Thr Gly
945                 950                 955                 960
Asn Phe Tyr Pro Gln Pro Leu Leu Asn Ser Ser Met Cys Leu Glu Asp
                965                 970                 975
Ser Arg Tyr Asn Trp Ser Phe Ile His Cys Pro Ala Cys Gln Cys Asn
            980                 985                 990
Gly His Ser Lys Cys Ile Asn Gln Ser Ile Cys Glu Lys Cys Glu Asn
        995                 1000                1005
Leu Thr Thr Gly Lys His Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly
    1010                1015                1020
Asp Pro Thr Asn Gly Gly Lys Cys Gln Pro Cys Lys Cys Asn Gly His
1025                1030                1035                1040
Ala Ser Leu Cys Asn Thr Asn Thr Gly Lys Cys Phe Cys Thr Thr Lys
                1045                1050                1055
Gly Val Lys Gly Asp Glu Cys Gln Leu Cys Glu Val Glu Asn Arg Tyr
            1060                1065                1070
Gln Gly Asn Pro Leu Arg Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp
        1075                1080                1085
Tyr Gln Phe Thr Phe Ser Leu Ser Gln Glu Asp Asp Arg Tyr Tyr Thr
    1090                1095                1100
Ala Ile Asn Phe Val Ala Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp
1105                1110                1115                1120
Met Phe Ile Asn Ala Ser Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala
                1125                1130                1135
Ala Ser Phe Ser Ala Gly Thr Gln Ala Gly Glu Glu Met Pro Val Val
            1140                1145                1150
Ser Lys Thr Asn Ile Lys Glu Tyr Lys Asp Ser Phe Ser Asn Glu Lys
        1155                1160                1165
Phe Asp Phe Arg Asn His Pro Asn Ile Thr Phe Phe Val Tyr Val Ser
    1170                1175                1180
Asn Phe Thr Trp Pro Ile Lys Ile Gln Ile Ala Phe Ser Gln His Ser
1185                1190                1195                1200
Asn Phe Met Asp Leu Val Gln Phe Phe Val Thr Phe Phe Ser Cys Phe
                1205                1210                1215
Leu Ser Leu Leu Leu Val Ala Ala Val Val Trp Lys Ile Lys Gln Ser
            1220                1225                1230
Cys Trp Ala Ser Arg Arg Arg Glu Gln Leu Leu Arg Glu Met Gln Gln
        1235                1240                1245
Met Ala Ser Arg Pro Phe Ala Ser Val Asn Val Ala Leu Glu Thr Asp
    1250                1255                1260
Glu Glu Pro Pro Asp Leu Ile Gly Gly Ser Ile Lys Thr Val Pro Lys
1265                1270                1275                1280
Pro Ile Ala Leu Glu Pro Cys Phe Gly Asn Lys Ala Ala Val Leu Ser
                1285                1290                1295
Val Phe Val Arg Leu Pro Arg Gly Leu Gly Gly Ile Pro Pro Pro Gly
            1300                1305                1310
Gln Ser Gly Leu Ala Val Ala Ser Ala Leu Val Asp Ile Ser Gln Gln
        1315                1320                1325
Met Pro Ile Val Tyr Lys Glu Lys Ser Gly Ala Val Arg Asn Arg Lys
    1330                1335                1340
Gln Gln Pro Pro Ala Gln Pro Gly Thr Cys Ile
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<213> Homo sapiens

<220>
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<400> 11

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gcg gcg acg gca gcg ctc gcg ggc agg agc ggc ggg ccg cac tgt gtc 96
Ala Ala Thr Ala Ala Leu Ala Gly Arg Ser Gly Gly Pro His Cys Val
20 25 30

aac ggc ggt cgc tgc aac cct ggc acc ggc cag tgc gtc tgc ccc gcc 144
Asn Gly Gly Arg Cys Asn Pro Gly Thr Gly Gln Cys Val Cys Pro Ala
35 40 45

ggc tgg gtg ggc gag caa tgc cag cac tgc ggg ggc cgc ttc aga cta 192
Gly Trp Val Gly Glu Gln Cys Gln His Cys Gly Gly Arg Phe Arg Leu
50 55 60

act gga tct tct ggg ttt gtg aca gat gga cct gga aat tat aaa tac 240
Thr Gly Ser Ser Gly Phe Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr
65 70 75 80

aaa acg aag tgc acg tgg ctc att gaa gga cag cca aat aga ata atg 288
Lys Thr Lys Cys Thr Trp Leu Ile Glu Gly Gln Pro Asn Arg Ile Met
85 90 95

aga ctt cgt ttc aat cat ttt gct aca gag tgt agt tgg gac cat tta 336
Arg Leu Arg Phe Asn His Phe Ala Thr Glu Cys Ser Trp Asp His Leu
100 105 110

tat gtt tat gat ggg gac tca att tat gca ccg cta gtt gct gca ttt 384
Tyr Val Tyr Asp Gly Asp Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe
115 120 125

agt ggc ctc att gtt cct gag aga gat ggc aat gag act gtc cct gag 432
Ser Gly Leu Ile Val Pro Glu Arg Asp Gly Asn Glu Thr Val Pro Glu
130 135 140

gtt gtt gcc aca tca ggt tat gcc ttg ctg cat ttt ttt agt gat gct 480
Val Val Ala Thr Ser Gly Tyr Ala Leu Leu His Phe Phe Ser Asp Ala
145 150 155 160

gct tat aat ttg act gga ttt aat att act tac agt ttt gat atg tgt 528
Ala Tyr Asn Leu Thr Gly Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys
165 170 175

cca aat aac tgc tca ggc cga gga gag tgt aag atc agt aat agc agc 576
Pro Asn Asn Cys Ser Gly Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser
180 185 190

gat act gtt gaa tgt gaa tgt tct gaa aac tgg aaa ggt gaa gca tgt 624
Asp Thr Val Glu Cys Glu Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys
195 200 205

gac att cct cac tgt aca gac aac tgt ggt ttt cct cat cga ggc atc 672
Asp Ile Pro His Cys Thr Asp Asn Cys Gly Phe Pro His Arg Gly Ile
210 215 220

tgc aat tca agt gat gtc aga gga tgc tcc tgc ttc tca gac tgg cag 720
Cys Asn Ser Ser Asp Val Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln
225 230 235 240

ggt cct gga tgt tca gtt cct gta cca gct aac cag tca ttt tgg act 768
Gly Pro Gly Cys Ser Val Pro Val Pro Ala Asn Gln Ser Phe Trp Thr
245 250 255

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cga gag gaa tat tct aac tta aag ctc ccc aga gca tct cat aaa gct      816
Arg Glu Glu Tyr Ser Asn Leu Lys Leu Pro Arg Ala Ser His Lys Ala
            260                 265                 270

gtg gtc aat gga aac att atg tgg gtt gtt gga gga tat atg ttc aac      864
Val Val Asn Gly Asn Ile Met Trp Val Val Gly Gly Tyr Met Phe Asn
        275                 280                 285

cac tca gat tat aac atg gtt cta gcg tat gac ctt gct tct agg gag      912
His Ser Asp Tyr Asn Met Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu
    290                 295                 300

tgg ctt cca cta aac cgt tct gtg aac aat gtg gtt gtt aga tat ggt      960
Trp Leu Pro Leu Asn Arg Ser Val Asn Asn Val Val Val Arg Tyr Gly
305                 310                 315                 320

cat tct ttg gca tta tac aag gat aaa att tac atg tat gga gga aaa     1008
His Ser Leu Ala Leu Tyr Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys
                325                 330                 335

att gat tca act ggg aat gtg acc aat gag ttg aga gtt ttt cac att     1056
Ile Asp Ser Thr Gly Asn Val Thr Asn Glu Leu Arg Val Phe His Ile
            340                 345                 350

cat aat gag tca tgg gtg ttg ttg acc cct aag gca aag gag cag tat     1104
His Asn Glu Ser Trp Val Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr
        355                 360                 365

gca gtg gtt ggg cac tct gca cac att gtt aca ctg aag aat ggc cga     1152
Ala Val Val Gly His Ser Ala His Ile Val Thr Leu Lys Asn Gly Arg
    370                 375                 380

gtg gtc atg ctg gtc atc ttt ggt cac tgc cct ctc tat gga tat ata     1200
Val Val Met Leu Val Ile Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile
385                 390                 395                 400

agc aat gtg cag gaa tat gat ttg gat aag aac aca tgg agt ata tta     1248
Ser Asn Val Gln Glu Tyr Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu
                405                 410                 415

cac acc cag ggt gcc ctt gtg caa ggg ggt tac ggc cat agc agt gtt     1296
His Thr Gln Gly Ala Leu Val Gln Gly Gly Tyr Gly His Ser Ser Val
            420                 425                 430

tac gac cat agg acc agg gcc cta tac gtt cat ggt ggc tac aag gct     1344
Tyr Asp His Arg Thr Arg Ala Leu Tyr Val His Gly Gly Tyr Lys Ala
        435                 440                 445

ttc agt gcc aat aag tac cgg ctt gca gat gat ctc tac cga tat gat     1392
Phe Ser Ala Asn Lys Tyr Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp
    450                 455                 460

gtg gat acc cag atg tgg acc att ctt aag gac agc cga ttt ttc cgt     1440
Val Asp Thr Gln Met Trp Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg
465                 470                 475                 480

tac ttg cac aca gct gtg ata gtg agt gga acc atg ctg gtg ttt ggg     1488
Tyr Leu His Thr Ala Val Ile Val Ser Gly Thr Met Leu Val Phe Gly
                485                 490                 495

gga aac aca cac aat gac aca tct atg agc cat ggc gcc aaa tgc ttc     1536
Gly Asn Thr His Asn Asp Thr Ser Met Ser His Gly Ala Lys Cys Phe
            500                 505                 510

tct tca gat ttc atg gcc tat gac att gcc tgt gac cgc tgg tca gtg     1584
Ser Ser Asp Phe Met Ala Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val
        515                 520                 525
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ctt ccc aga cct gat ctc cac cat gat gtc aac aga ttt ggc cat tca      1632
Leu Pro Arg Pro Asp Leu His His Asp Val Asn Arg Phe Gly His Ser
    530                 535                 540

gca gtc tta cac aac agc acc atg tat gtg ttc ggt ggt ttc aat agt      1680
Ala Val Leu His Asn Ser Thr Met Tyr Val Phe Gly Gly Phe Asn Ser
545                 550                 555                 560

ctc ctc ctc agc gac atc ctg gta ttc acc tcg gaa cag tgt gat gcg      1728
Leu Leu Leu Ser Asp Ile Leu Val Phe Thr Ser Glu Gln Cys Asp Ala
                565                 570                 575

cat cgg agt gaa gcc gct tgt tta gca gca gga cct ggt att cgg tgt      1776
His Arg Ser Glu Ala Ala Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys
            580                 585                 590

gtg tgg aac aca ggg tcg tct cag tgt atc tcg tgg gcg ctg gca act      1824
Val Trp Asn Thr Gly Ser Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr
        595                 600                 605

gat gaa caa gaa gaa aag tta aaa tca gaa tgt ttt tcc aaa aga act      1872
Asp Glu Gln Glu Glu Lys Leu Lys Ser Glu Cys Phe Ser Lys Arg Thr
    610                 615                 620

ctt gac cat gac aga tgt gac cag cac aca gat tgt tac agc tgc aca      1920
Leu Asp His Asp Arg Cys Asp Gln His Thr Asp Cys Tyr Ser Cys Thr
625                 630                 635                 640

gcc aac acc aat gac tgc cac tgg tgc aat gac cat tgt gtc ccc agg      1968
Ala Asn Thr Asn Asp Cys His Trp Cys Asn Asp His Cys Val Pro Arg
                645                 650                 655

aac cac agc tgc tca gaa ggc cag atc tcc att ttt agg tat gag aat      2016
Asn His Ser Cys Ser Glu Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn
            660                 665                 670

tgc ccc aag gat aac cct atg tac tac tgt aac aag aag acc agc tgc      2064
Cys Pro Lys Asp Asn Pro Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys
        675                 680                 685

agg agc tgt gcc ctg gac cag aac tgc cag tgg gag ccc cgg aat cag      2112
Arg Ser Cys Ala Leu Asp Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln
    690                 695                 700

gag tgc att gcc ctg ccc gaa aat atc tgt ggc att ggc tgg cat ttg      2160
Glu Cys Ile Ala Leu Pro Glu Asn Ile Cys Gly Ile Gly Trp His Leu
705                 710                 715                 720

gtt gga aac tca tgt ttg aaa att act act gcc aag gag aat tat gac      2208
Val Gly Asn Ser Cys Leu Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp
                725                 730                 735

aat gct aaa ttg ttc tgt agg aac cac aat gcc ctt ttg gct tct ctt      2256
Asn Ala Lys Leu Phe Cys Arg Asn His Asn Ala Leu Leu Ala Ser Leu
            740                 745                 750

aca acc cag aag aag gta gaa ttt gtc ctt aag cag ctg cga ata atg      2304
Thr Thr Gln Lys Lys Val Glu Phe Val Leu Lys Gln Leu Arg Ile Met
        755                 760                 765

cag tca tct cag agc atg tcc aag ctc acc tta acc cca tgg gtc ggc      2352
Gln Ser Ser Gln Ser Met Ser Lys Leu Thr Leu Thr Pro Trp Val Gly
    770                 775                 780
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ctt cgg aag atc aat gtg tcc tac tgg tgc tgg gaa gat atg tcc cca      2400
Leu Arg Lys Ile Asn Val Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro
785                 790                 795                 800

ttt aca aat agt tta cta cag tgg atg ccg tct gag ccc agt gat gct      2448
Phe Thr Asn Ser Leu Leu Gln Trp Met Pro Ser Glu Pro Ser Asp Ala
                805                 810                 815

gga ttc tgt gga att tta tca gaa ccc agt act cgg gga ctg aag gct      2496
Gly Phe Cys Gly Ile Leu Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala
        820                 825                 830

gca acc tgc atc aac cca ctc aat ggt agt gtc tgt gaa agg cct gca      2544
Ala Thr Cys Ile Asn Pro Leu Asn Gly Ser Val Cys Glu Arg Pro Ala
    835                 840                 845

aac cac agt gct aag cag tgc cgg aca cca tgt gcc ttg agg aca gca      2592
Asn His Ser Ala Lys Gln Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala
850                 855                 860

tgt gga gat tgc acc agc ggc agc tct gag tgc atg tgg tgc agc aac      2640
Cys Gly Asp Cys Thr Ser Gly Ser Ser Glu Cys Met Trp Cys Ser Asn
865                 870                 875                 880

atg aag cag tgt gtg gac tcc aat gcc tat gtg gcc tcc ttc cct ttt      2688
Met Lys Gln Cys Val Asp Ser Asn Ala Tyr Val Ala Ser Phe Pro Phe
                885                 890                 895

ggc cag tgt atg gaa tgg tat acg atg agc acc tgc ccc cct gaa aat      2736
Gly Gln Cys Met Glu Trp Tyr Thr Met Ser Thr Cys Pro Pro Glu Asn
            900                 905                 910

tgt tca ggc tac tgt acc tgt agt cat tgc ttg gag caa cca ggc tgt      2784
Cys Ser Gly Tyr Cys Thr Cys Ser His Cys Leu Glu Gln Pro Gly Cys
        915                 920                 925

ggc tgg tgt act gat ccc agc aat act ggc aaa ggg aaa tgc ata gag      2832
Gly Trp Cys Thr Asp Pro Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu
    930                 935                 940

ggt tcc tat aaa gga cca gtg aag atg cct tcg caa gcc cct aca gga      2880
Gly Ser Tyr Lys Gly Pro Val Lys Met Pro Ser Gln Ala Pro Thr Gly
945                 950                 955                 960

aat ttc tat cca cag ccc ctg ctc aat tcc agc atg tgt cta gag gac      2928
Asn Phe Tyr Pro Gln Pro Leu Leu Asn Ser Ser Met Cys Leu Glu Asp
                965                 970                 975

agc aga tac aac tgg tct ttc att cac tgt cca gct tgc caa tgc aac      2976
Ser Arg Tyr Asn Trp Ser Phe Ile His Cys Pro Ala Cys Gln Cys Asn
            980                 985                 990

ggc cac agt aaa tgc atc aat cag agc atc tgt gag aag tgt gag aac      3024
Gly His Ser Lys Cys Ile Asn Gln Ser Ile Cys Glu Lys Cys Glu Asn
        995                1000                1005

ctg acc aca ggc aag cac tgc gag acc tgc ata tct ggc ttc tac ggt      3072
Leu Thr Thr Gly Lys His Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly
   1010                1015                1020

gat ccc acc aat gga ggg aaa tgt cag cca tgc aag tgc aat ggg cac      3120
Asp Pro Thr Asn Gly Gly Lys Cys Gln Pro Cys Lys Cys Asn Gly His
1025               1030                1035                1040

gcg tct ctg tgc aac acc aac acg ggc aag tgc ttc tgc acc acc aag      3168
Ala Ser Leu Cys Asn Thr Asn Thr Gly Lys Cys Phe Cys Thr Thr Lys
               1045                1050                1055
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ggc gtc aag ggg gac gag tgc cag cta tgt gag gta gaa aat cga tac     3216
Gly Val Lys Gly Asp Glu Cys Gln Leu Cys Glu Val Glu Asn Arg Tyr
            1060                1065                1070

caa gga aac cct ctc aga gga aca tgt tat tat act ctt ctt att gac     3264
Gln Gly Asn Pro Leu Arg Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp
        1075                1080                1085

tat cag ttc acc ttt agt cta tcc cag gaa gat gat cgc tat tac aca     3312
Tyr Gln Phe Thr Phe Ser Leu Ser Gln Glu Asp Asp Arg Tyr Tyr Thr
    1090                1095                1100

gct atc aat ttt gtg gct act cct gac gaa caa aac agg gat ttg gac     3360
Ala Ile Asn Phe Val Ala Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp
1105                1110                1115                1120

atg ttc atc aat gcc tcc aag aat ttc aac ctc aac atc acc tgg gct     3408
Met Phe Ile Asn Ala Ser Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala
                1125                1130                1135

gcc agt ttc tca gct gga acc cag gct gga gaa gag atg cct gtt gtt     3456
Ala Ser Phe Ser Ala Gly Thr Gln Ala Gly Glu Glu Met Pro Val Val
            1140                1145                1150

tca aaa acc aac att aag gag tac aaa gat agt ttc tct aat gag aag     3504
Ser Lys Thr Asn Ile Lys Glu Tyr Lys Asp Ser Phe Ser Asn Glu Lys
        1155                1160                1165

ttt gat ttt cgc aac cac cca aat atc act ttc ttt gtt tat gtc agt     3552
Phe Asp Phe Arg Asn His Pro Asn Ile Thr Phe Phe Val Tyr Val Ser
    1170                1175                1180

aat ttc acc tgg ccc atc aaa att cag att gcc ttc tct cag cac agc     3600
Asn Phe Thr Trp Pro Ile Lys Ile Gln Ile Ala Phe Ser Gln His Ser
1185                1190                1195                1200

aat ttt atg gac ctg gta cag ttc ttc gtg act ttc ttc agt tgt ttc     3648
Asn Phe Met Asp Leu Val Gln Phe Phe Val Thr Phe Phe Ser Cys Phe
                1205                1210                1215

ctc tct ttg ctc ctg gtg gct gct gtg gtt tgg aag atc aaa caa agt     3696
Leu Ser Leu Leu Leu Val Ala Ala Val Val Trp Lys Ile Lys Gln Ser
            1220                1225                1230

tgt tgg gcc tcc aga cgt aga gag caa ctt ctt cga gag atg caa cag     3744
Cys Trp Ala Ser Arg Arg Arg Glu Gln Leu Leu Arg Glu Met Gln Gln
        1235                1240                1245

atg gcc agc cgt ccc ttt gcc tct gta aat gtc gcc ttg gaa aca gat     3792
Met Ala Ser Arg Pro Phe Ala Ser Val Asn Val Ala Leu Glu Thr Asp
    1250                1255                1260

gag gag cct cct gat ctt att ggg ggg agt ata aag act gtt ccc aaa     3840
Glu Glu Pro Pro Asp Leu Ile Gly Gly Ser Ile Lys Thr Val Pro Lys
1265                1270                1275                1280

ccc att gca ctg gag ccg tgt ttt ggc aac aaa gcc gct gtc ctc tct     3888
Pro Ile Ala Leu Glu Pro Cys Phe Gly Asn Lys Ala Ala Val Leu Ser
                1285                1290                1295

gtg ttt gtg agg ctc cct cga ggc ctg ggt ggc atc cct cct cct ggg     3936
Val Phe Val Arg Leu Pro Arg Gly Leu Gly Gly Ile Pro Pro Pro Gly
            1300                1305                1310

cag tca ggt ctt gct gtg gcc agc gcc ctg gtg gac att tct cag cag     3984
Gln Ser Gly Leu Ala Val Ala Ser Ala Leu Val Asp Ile Ser Gln Gln
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atg ccg ata gtg tac aag gag aag tca gga gcc gtg aga aac cgg aag 4032
Met Pro Ile Val Tyr Lys Glu Lys Ser Gly Ala Val Arg Asn Arg Lys
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cag cag ccc cct gca cag cct ggg acc tgc atc tga 4068
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35 40 45
Leu Pro Arg Leu Leu Ser Pro Pro Leu Arg Pro Arg Leu Leu Leu Leu
50 55 60
Leu Leu Leu Leu Pro Pro Pro Leu Leu Leu Leu Leu Leu Pro Cys Glu
65 70 75 80
Ala Glu Ala Ala Ala Ala Ala Ala Ala Val Ser Gly Ser Ala Ala Ala
85 90 95
Glu Ala Lys Glu Cys Asp Arg Pro Cys Val Asn Gly Gly Arg Cys Asn
100 105 110
Pro Gly Thr Gly Gln Cys Val Cys Pro Ala Gly Trp Val Gly Glu Gln
115 120 125
Cys Gln His Cys Gly Gly Arg Phe Arg Leu Thr Gly Ser Ser Gly Phe
130 135 140
Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr Lys Thr Lys Cys Thr Trp
145 150 155 160
Leu Ile Glu Gly Gln Pro Asn Arg Ile Met Arg Leu Arg Phe Asn His
165 170 175
Phe Ala Thr Glu Cys Ser Trp Asp His Leu Tyr Val Tyr Asp Gly Asp
180 185 190
Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe Ser Gly Leu Ile Val Pro
195 200 205
Glu Arg Asp Gly Asn Glu Thr Val Pro Glu Val Val Ala Thr Ser Gly
210 215 220
Tyr Ala Leu Leu His Phe Phe Ser Asp Ala Ala Tyr Asn Leu Thr Gly
225 230 235 240
Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys Pro Asn Asn Cys Ser Gly
245 250 255
Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser Glu Thr Val Glu Cys Glu
260 265 270
Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys Asp Ile Pro His Cys Thr
275 280 285
Asp Asn Cys Gly Phe Pro His Arg Gly Ile Cys Asn Ser Ser Asp Val
290 295 300
Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln Gly Pro Gly Cys Ser Val
305 310 315 320
Pro Val Pro Ala Asn Gln Ser Phe Trp Thr Arg Glu Glu Tyr Ser Asn
325 330 335
Leu Lys Leu Pro Arg Ala Ser His Lys Ala Val Val Asn Gly Asn Ile
340 345 350
Met Trp Val Val Gly Gly Tyr Met Phe Asn His Ser Asp Tyr Asn Met
355 360 365
Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu Trp Leu Pro Leu Asn Arg
370 375 380
Ser Val Asn Asn Val Val Val Arg Tyr Gly His Ser Leu Ala Leu Tyr
385 390 395 400

Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys Ile Asp Pro Thr Gly Asn
405 410 415
Val Thr Asn Glu Leu Arg Val Phe His Ile His Asn Glu Ser Trp Val
420 425 430
Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr Ala Val Val Gly His Ser
435 440 445
Ala His Ile Val Thr Leu Lys Asn Gly Arg Val Val Met Leu Val Ile
450 455 460
Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile Ser Asn Val Gln Glu Tyr
465 470 475 480
Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu His Thr Gln Gly Ala Leu
485 490 495
Val Gln Gly Gly Tyr Gly His Ser Ser Val Tyr Asp His Arg Thr Arg
500 505 510
Ala Leu Tyr Val His Gly Gly Tyr Lys Ala Phe Ser Ala Asn Lys Tyr
515 520 525
Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp Val Asp Thr Gln Met Trp
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Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg Tyr Leu His Thr Ala Val
545 550 555 560
Ile Val Ser Gly Thr Met Leu Val Phe Gly Gly Asn Thr His Asn Asp
565 570 575
Thr Ser Met Ser His Gly Ala Lys Cys Phe Ser Ser Asp Phe Met Ala
580 585 590
Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val Leu Pro Arg Pro Asp Leu
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His His Asp Val Asn Arg Phe Gly His Ser Ala Val Leu His Asn Ser
610 615 620
Thr Met Tyr Val Phe Gly Gly Phe Asn Ser Leu Leu Leu Ser Asp Ile
625 630 635 640
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Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys Val Trp Asn Thr Gly Ser
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Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr Asp Glu Gln Glu Glu Lys
675 680 685
Leu Lys Ser Glu Cys Phe Ser Lys Arg Thr Leu Asp His Asp Arg Cys
690 695 700
Asp Gln His Thr Asp Cys Tyr Ser Cys Thr Ala Asn Thr Asn Asp Cys
705 710 715 720
His Trp Cys Asn Asp His Cys Val Pro Arg Asn His Ser Cys Ser Glu
725 730 735
Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn Cys Pro Lys Asp Asn Pro
740 745 750
Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys Arg Ser Cys Ala Leu Asp
755 760 765
Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln Glu Cys Ile Ala Leu Pro
770 775 780
Glu Asn Ile Cys Gly Ile Gly Trp His Leu Val Gly Asn Ser Cys Leu
785 790 795 800
Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp Asn Ala Lys Leu Phe Cys
805 810 815
Arg Asn His Asn Ala Leu Leu Ala Ser Leu Thr Thr Gln Lys Lys Val
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850 855 860
Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro Phe Thr Asn Ser Leu Leu
865 870 875 880
Gln Trp Met Pro Ser Glu Pro Ser Asp Ala Gly Phe Cys Gly Ile Leu
885 890 895
Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala Ala Thr Cys Ile Asn Pro
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915 920 925

Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala Cys Gly Asp Cys Thr Ser
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Gly Ser Ser Glu Cys Met Trp Cys Ser Asn Met Lys Gln Cys Val Asp
945 950 955 960
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980 985 990
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Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu Gly Ser Tyr Lys Gly Pro
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Phe Ile His Cys Pro Ala Cys Gln Cys Asn Gly His Ser Lys Cys Ile
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Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly Asp Pro Thr Asn Gly Gly
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Lys Cys Gln Pro Cys Lys Cys Asn Gly His Ala Ser Leu Cys Asn Thr
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Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp Tyr Gln Phe Thr Phe Ser
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1170 1175 1180
Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp Met Phe Ile Asn Ala Ser
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Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala Ala Ser Phe Ser Ala Gly
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Pro Asn Ile Thr Phe Phe Val Tyr Val Ser Asn Phe Thr Trp Pro Ile
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Gln Phe Phe Val Thr Phe Phe Ser Cys Phe Leu Ser Leu Leu Leu Val
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Ala Ser Val Asn Val Ala Leu Glu Thr Asp Glu Glu Pro Pro Asp Leu
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Ile Gly Gly Ser Ile Lys Thr Val Pro Lys Pro Ile Ala Leu Glu Pro
1345 1350 1355 1360
Cys Phe Gly Asn Lys Ala Ala Val Leu Ser Val Phe Val Arg Leu Pro
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gcg gcg acg gca gcg ctc gcg ggc agg agc ggc ggg ccg cac tgg gac      96
Ala Ala Thr Ala Ala Leu Ala Gly Arg Ser Gly Gly Pro His Trp Asp
            20                  25                  30

tgg gac gtg acc agg gct ggg agg ccg ggg ctg ggg gcc ggg ctg cgc     144
Trp Asp Val Thr Arg Ala Gly Arg Pro Gly Leu Gly Ala Gly Leu Arg
        35                  40                  45

ctc ccg cgg ctg ctg tct cca ccg ctg cgg cca cgg ctg ctg ctg ctg     192
Leu Pro Arg Leu Leu Ser Pro Pro Leu Arg Pro Arg Leu Leu Leu Leu
    50                  55                  60

ctg ttg ttg ctc ccg ccg ccg ctg ttg ctg ctg ctg ctg ccc tgt gag     240
Leu Leu Leu Leu Pro Pro Pro Leu Leu Leu Leu Leu Leu Pro Cys Glu
65                  70                  75                  80

gcc gag gcc gcg gcg gcg gcg gcg gcg gtg tcg ggc tca gcc gca gcc     288
Ala Glu Ala Ala Ala Ala Ala Ala Ala Val Ser Gly Ser Ala Ala Ala
                85                  90                  95

gag gcc aag gaa tgt gac cgg ccc tgt gtc aac ggc ggt cgc tgc aac     336
Glu Ala Lys Glu Cys Asp Arg Pro Cys Val Asn Gly Gly Arg Cys Asn
            100                 105                 110

cct ggc acc ggc cag tgc gtc tgc ccc gcc ggc tgg gtg ggc gag caa     384
Pro Gly Thr Gly Gln Cys Val Cys Pro Ala Gly Trp Val Gly Glu Gln
        115                 120                 125

tgc cag cac tgc ggg ggc cgc ttc aga cta act gga tct tct ggg ttt     432
Cys Gln His Cys Gly Gly Arg Phe Arg Leu Thr Gly Ser Ser Gly Phe
    130                 135                 140

gtg aca gat gga cct gga aat tat aaa tac aaa acg aag tgc acg tgg     480
Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr Lys Thr Lys Cys Thr Trp
145                 150                 155                 160

ctc att gaa gga cag cca aat aga ata atg aga ctt cgt ttc aat cat     528
Leu Ile Glu Gly Gln Pro Asn Arg Ile Met Arg Leu Arg Phe Asn His
                165                 170                 175

ttt gct aca gag tgt agt tgg gac cat tta tat gtt tat gat ggg gac     576
Phe Ala Thr Glu Cys Ser Trp Asp His Leu Tyr Val Tyr Asp Gly Asp
            180                 185                 190

tca att tat gca ccg cta gtt gct gca ttt agt ggc ctc att gtt cct     624
Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe Ser Gly Leu Ile Val Pro
        195                 200                 205

gag aga gat ggc aat gag act gtc cct gag gtt gtt gcc aca tca ggt     672
Glu Arg Asp Gly Asn Glu Thr Val Pro Glu Val Val Ala Thr Ser Gly
    210                 215                 220

tat gcc ttg ctg cat ttt ttt agt gat gct gct tat aat ttg act gga     720
Tyr Ala Leu Leu His Phe Phe Ser Asp Ala Ala Tyr Asn Leu Thr Gly
225                 230                 235                 240
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ttt aat att act tac agt ttt gat atg tgt cca aat aac tgc tca ggc      768
Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys Pro Asn Asn Cys Ser Gly
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cga gga gag tgt aag atc agt aat agc agc gaa act gtt gaa tgt gaa      816
Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser Glu Thr Val Glu Cys Glu
            260                 265                 270

tgt tct gaa aac tgg aaa ggt gaa gca tgt gac att cct cac tgt aca      864
Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys Asp Ile Pro His Cys Thr
        275                 280                 285

gac aac tgt ggt ttt cct cat cga ggc atc tgc aat tca agt gat gtc      912
Asp Asn Cys Gly Phe Pro His Arg Gly Ile Cys Asn Ser Ser Asp Val
    290                 295                 300

aga gga tgc tcc tgc ttc tca gac tgg cag ggt cct gga tgt tca gtt      960
Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln Gly Pro Gly Cys Ser Val
305                 310                 315                 320

cct gta cca gct aac cag tca ttt tgg act cga gag gaa tat tct aac     1008
Pro Val Pro Ala Asn Gln Ser Phe Trp Thr Arg Glu Glu Tyr Ser Asn
                325                 330                 335

tta aag ctc ccc aga gca tct cat aaa gct gtg gtc aat gga aac att     1056
Leu Lys Leu Pro Arg Ala Ser His Lys Ala Val Val Asn Gly Asn Ile
            340                 345                 350

atg tgg gtt gtt gga gga tat atg ttc aac cac tca gat tat aac atg     1104
Met Trp Val Val Gly Gly Tyr Met Phe Asn His Ser Asp Tyr Asn Met
        355                 360                 365

gtt cta gcg tat gac ctt gct tct agg gag tgg ctt cca cta aac cgt     1152
Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu Trp Leu Pro Leu Asn Arg
    370                 375                 380

tct gtg aac aat gtg gtt gtt aga tat ggt cat tct ttg gca tta tac     1200
Ser Val Asn Asn Val Val Val Arg Tyr Gly His Ser Leu Ala Leu Tyr
385                 390                 395                 400

aag gat aaa att tac atg tat gga gga aaa att gat cca act ggg aat     1248
Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys Ile Asp Pro Thr Gly Asn
                405                 410                 415

gtg acc aat gag ttg aga gtt ttt cac att cat aat gag tca tgg gtg     1296
Val Thr Asn Glu Leu Arg Val Phe His Ile His Asn Glu Ser Trp Val
            420                 425                 430

ttg ttg acc cct aag gca aag gag cag tat gca gtg gtt ggg cac tct     1344
Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr Ala Val Val Gly His Ser
        435                 440                 445

gca cac att gtt aca ctg aag aat ggc cga gtg gtc atg ctg gtc atc     1392
Ala His Ile Val Thr Leu Lys Asn Gly Arg Val Val Met Leu Val Ile
    450                 455                 460

ttt ggt cac tgc cct ctc tat gga tat ata agc aat gtg cag gaa tat     1440
Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile Ser Asn Val Gln Glu Tyr
465                 470                 475                 480

gat ttg gat aag aac aca tgg agt ata tta cac acc cag ggt gcc ctt     1488
Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu His Thr Gln Gly Ala Leu
                485                 490                 495
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gtg caa ggg ggt tac ggc cat agc agt gtt tac gac cat agg acc agg      1536
Val Gln Gly Gly Tyr Gly His Ser Ser Val Tyr Asp His Arg Thr Arg
            500                 505                 510

gcc cta tac gtt cat ggt ggc tac aag gct ttc agt gcc aat aag tac      1584
Ala Leu Tyr Val His Gly Gly Tyr Lys Ala Phe Ser Ala Asn Lys Tyr
        515                 520                 525

cgg ctt gca gat gat ctc tac cga tat gat gtg gat acc cag atg tgg      1632
Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp Val Asp Thr Gln Met Trp
    530                 535                 540

acc att ctt aag gac agc cga ttt ttc cgt tac ttg cac aca gct gtg      1680
Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg Tyr Leu His Thr Ala Val
545                 550                 555                 560

ata gtg agt gga acc atg ctg gtg ttt ggg gga aac aca cac aat gac      1728
Ile Val Ser Gly Thr Met Leu Val Phe Gly Gly Asn Thr His Asn Asp
                565                 570                 575

aca tct atg agc cat ggc gcc aaa tgc ttc tct tca gat ttc atg gcc      1776
Thr Ser Met Ser His Gly Ala Lys Cys Phe Ser Ser Asp Phe Met Ala
            580                 585                 590

tat gac att gcc tgt gac cgc tgg tca gtg ctt ccc aga cct gat ctc      1824
Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val Leu Pro Arg Pro Asp Leu
        595                 600                 605

cac cat gat gtc aac aga ttt ggc cat tca gca gtc tta cac aac agc      1872
His His Asp Val Asn Arg Phe Gly His Ser Ala Val Leu His Asn Ser
    610                 615                 620

acc atg tat gtg ttc ggt ggt ttc aat agt ctc ctc ctc agc gac atc      1920
Thr Met Tyr Val Phe Gly Gly Phe Asn Ser Leu Leu Leu Ser Asp Ile
625                 630                 635                 640

ctg gta ttc acc tcg gaa cag tgt gat gcg cat cgg agt gaa gcc gct      1968
Leu Val Phe Thr Ser Glu Gln Cys Asp Ala His Arg Ser Glu Ala Ala
                645                 650                 655

tgt tta gca gca gga cct ggt att cgg tgt gtg tgg aac aca ggg tcg      2016
Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys Val Trp Asn Thr Gly Ser
            660                 665                 670

tct cag tgt atc tcg tgg gcg ctg gca act gat gaa caa gaa gaa aag      2064
Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr Asp Glu Gln Glu Glu Lys
        675                 680                 685

tta aaa tca gaa tgt ttt tcc aaa aga act ctt gac cat gac aga tgt      2112
Leu Lys Ser Glu Cys Phe Ser Lys Arg Thr Leu Asp His Asp Arg Cys
    690                 695                 700

gac cag cac aca gat tgt tac agc tgt aca gcc aac acc aat gac tgc      2160
Asp Gln His Thr Asp Cys Tyr Ser Cys Thr Ala Asn Thr Asn Asp Cys
705                 710                 715                 720

cac tgg tgc aat gac cat tgt gtc ccc agg aac cac agc tgc tca gaa      2208
His Trp Cys Asn Asp His Cys Val Pro Arg Asn His Ser Cys Ser Glu
                725                 730                 735

ggc cag atc tcc att ttt agg tat gag aat tgc ccc aag gat aac ccc      2256
Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn Cys Pro Lys Asp Asn Pro
            740                 745                 750

atg tac tac tgt aac aag aag acc agc tgc agg agc tgt gcc ctg gac      2304
Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys Arg Ser Cys Ala Leu Asp
        755                 760                 765
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cag aac tgc cag tgg gag ccc cgg aat cag gag tgc att gcc ctg ccc     2352
Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln Glu Cys Ile Ala Leu Pro
    770                 775                 780

gaa aat atc tgt ggc att ggc tgg cat ttg gtt gga aac tca tgt ttg     2400
Glu Asn Ile Cys Gly Ile Gly Trp His Leu Val Gly Asn Ser Cys Leu
785                 790                 795                 800

aaa att act act gcc aag gag aat tat gac aat gct aaa ttg ttc tgt     2448
Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp Asn Ala Lys Leu Phe Cys
                805                 810                 815

agg aac cac aat gcc ctt ttg gct tct ctt aca acc cag aag aag gta     2496
Arg Asn His Asn Ala Leu Leu Ala Ser Leu Thr Thr Gln Lys Lys Val
            820                 825                 830

gaa ttt gtc ctt aag cag ctg cga ata atg cag tca tct cag agc atg     2544
Glu Phe Val Leu Lys Gln Leu Arg Ile Met Gln Ser Ser Gln Ser Met
        835                 840                 845

tcc aag ctc acc tta acc cca tgg gtc ggc ctt cgg aag atc aat gtg     2592
Ser Lys Leu Thr Leu Thr Pro Trp Val Gly Leu Arg Lys Ile Asn Val
    850                 855                 860

tcc tac tgg tgc tgg gaa gat atg tcc cca ttt aca aat agt tta cta     2640
Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro Phe Thr Asn Ser Leu Leu
865                 870                 875                 880

cag tgg atg ccg tct gag ccc agt gat gct gga ttc tgt gga att tta     2688
Gln Trp Met Pro Ser Glu Pro Ser Asp Ala Gly Phe Cys Gly Ile Leu
                885                 890                 895

tca gaa ccc agt act cgg gga ctg aag gct gca acc tgc atc aac cca     2736
Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala Ala Thr Cys Ile Asn Pro
            900                 905                 910

ctc aat ggt agt gtc tgt gaa agg cct gca aac cac agt gct aag cag     2784
Leu Asn Gly Ser Val Cys Glu Arg Pro Ala Asn His Ser Ala Lys Gln
        915                 920                 925

tgc cgg aca cca tgt gcc ttg agg aca gca tgt gga gat tgc acc agc     2832
Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala Cys Gly Asp Cys Thr Ser
    930                 935                 940

ggc agc tct gag tgc atg tgg tgc agc aac atg aag cag tgt gtg gac     2880
Gly Ser Ser Glu Cys Met Trp Cys Ser Asn Met Lys Gln Cys Val Asp
945                 950                 955                 960

tcc aat gcc tat gtg gcc tcc ttc cct ttt ggc cag tgt atg gaa tgg     2928
Ser Asn Ala Tyr Val Ala Ser Phe Pro Phe Gly Gln Cys Met Glu Trp
                965                 970                 975

tat acg atg agc acc tgc ccc cct gaa aat tgt tca ggc tac tgt acc     2976
Tyr Thr Met Ser Thr Cys Pro Pro Glu Asn Cys Ser Gly Tyr Cys Thr
            980                 985                 990

tgt agt cat tgc ttg gag caa cca ggc tgt ggc tgg tgt act gat ccc     3024
Cys Ser His Cys Leu Glu Gln Pro Gly Cys Gly Trp Cys Thr Asp Pro
        995                 1000                1005

agc aat act ggc aaa ggg aaa tgc ata gag ggt tcc tat aaa gga cca     3072
Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu Gly Ser Tyr Lys Gly Pro
    1010                1015                1020
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gtg aag atg cct tcg caa gcc cct aca gga aat ttc tat cca cag ccc 3120
Val Lys Met Pro Ser Gln Ala Pro Thr Gly Asn Phe Tyr Pro Gln Pro
1025 1030 1035 1040

ctg ctc aat tcc agc atg tgt cta gag gac agc aga tac aac tgg tct 3168
Leu Leu Asn Ser Ser Met Cys Leu Glu Asp Ser Arg Tyr Asn Trp Ser
1045 1050 1055

ttc att cac tgt cca gct tgc caa tgc aac ggc cac agt aaa tgc atc 3216
Phe Ile His Cys Pro Ala Cys Gln Cys Asn Gly His Ser Lys Cys Ile
1060 1065 1070

aat cag agc atc tgt gag aag tgt gag aac ctg acc aca ggc aag cac 3264
Asn Gln Ser Ile Cys Glu Lys Cys Glu Asn Leu Thr Thr Gly Lys His
1075 1080 1085

tgc gag acc tgc ata tct ggc ttc tac ggt gat ccc acc aat gga ggg 3312
Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly Asp Pro Thr Asn Gly Gly
1090 1095 1100

aaa tgt cag cca tgc aag tgc aat ggg cac gcg tct ctg tgc aac acc 3360
Lys Cys Gln Pro Cys Lys Cys Asn Gly His Ala Ser Leu Cys Asn Thr
1105 1110 1115 1120

aac acg ggc aag tgc ttc tgc acc acc aag ggc gtc aag ggg gac gag 3408
Asn Thr Gly Lys Cys Phe Cys Thr Thr Lys Gly Val Lys Gly Asp Glu
1125 1130 1135

tgc cag cta tgt gag gta gaa aat cga tac caa gga aac cct ctc aga 3456
Cys Gln Leu Cys Glu Val Glu Asn Arg Tyr Gln Gly Asn Pro Leu Arg
1140 1145 1150

gga aca tgt tat tat act ctt ctt att gac tat cag ttc acc ttt agt 3504
Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp Tyr Gln Phe Thr Phe Ser
1155 1160 1165

cta tcc cag gaa gat gat cgc tat tac aca gct atc aat ttt gtg gct 3552
Leu Ser Gln Glu Asp Asp Arg Tyr Tyr Thr Ala Ile Asn Phe Val Ala
1170 1175 1180

act cct gac gaa caa aac agg gat ttg gac atg ttc atc aat gcc tcc 3600
Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp Met Phe Ile Asn Ala Ser
1185 1190 1195 1200

aag aat ttc aac ctc aac atc acc tgg gct gcc agt ttc tca gct gga 3648
Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala Ala Ser Phe Ser Ala Gly
1205 1210 1215

acc cag gct gga gaa gag atg cct gtt gtt tca aaa acc aac att aag 3696
Thr Gln Ala Gly Glu Glu Met Pro Val Val Ser Lys Thr Asn Ile Lys
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gag tac aaa gat agt ttc tct aat gag aag ttt gat ttt cgc aac cac 3744
Glu Tyr Lys Asp Ser Phe Ser Asn Glu Lys Phe Asp Phe Arg Asn His
1235 1240 1245

cca aat atc act ttc ttt gtt tat gtc agt aat ttc acc tgg ccc atc 3792
Pro Asn Ile Thr Phe Phe Val Tyr Val Ser Asn Phe Thr Trp Pro Ile
1250 1255 1260

aaa att cag att gcc ttc tct cag cac agc aat ttt atg gac ctg gta 3840
Lys Ile Gln Ile Ala Phe Ser Gln His Ser Asn Phe Met Asp Leu Val
1265 1270 1275 1280

cag ttc ttc gtg act ttc ttc agt tgt ttc ctc tct ttg ctc ctg gtg 3888
Gln Phe Phe Val Thr Phe Phe Ser Cys Phe Leu Ser Leu Leu Leu Val
1285 1290 1295

gct gct gtg gtt tgg aag atc aaa caa agt tgt tgg gcc tcc aga cgt 3936
Ala Ala Val Val Trp Lys Ile Lys Gln Ser Cys Trp Ala Ser Arg Arg
1300 1305 1310

aga gag caa ctt ctt cga gag atg caa cag atg gcc agc cgt ccc ttt 3984
Arg Glu Gln Leu Leu Arg Glu Met Gln Gln Met Ala Ser Arg Pro Phe
1315 1320 1325

gcc tct gta aat gtc gcc ttg gaa aca gat gag gag cct cct gat ctt 4032
Ala Ser Val Asn Val Ala Leu Glu Thr Asp Glu Glu Pro Pro Asp Leu
1330 1335 1340

att ggg ggg agt ata aag act gtt ccc aaa ccc att gca ctg gag ccg 4080
Ile Gly Gly Ser Ile Lys Thr Val Pro Lys Pro Ile Ala Leu Glu Pro
1345 1350 1355 1360

tgt ttt ggc aac aaa gcc gct gtc ctc tct gtg ttt gtg agg ctc cct 4128
Cys Phe Gly Asn Lys Ala Ala Val Leu Ser Val Phe Val Arg Leu Pro
1365 1370 1375

cga ggc ctg ggt ggc atc cct cct cct ggg cag tca ggt ctt gct gtg 4176
Arg Gly Leu Gly Gly Ile Pro Pro Pro Gly Gln Ser Gly Leu Ala Val
1380 1385 1390

gcc agc gcc ctg gtg gac att tct cag cag atg ccg ata gtg tac aag 4224
Ala Ser Ala Leu Val Asp Ile Ser Gln Gln Met Pro Ile Val Tyr Lys
1395 1400 1405

gag aag tca gga gcc gtg aga aac cgg aag cag cag ccc cct gca cag 4272
Glu Lys Ser Gly Ala Val Arg Asn Arg Lys Gln Gln Pro Pro Ala Gln
1410 1415 1420

cct ggg acc tgc atc tga 4290
Pro Gly Thr Cys Ile
1425

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Leu Met Ser Ala Gln Glu Ser Trp Ala
20 25

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             20                  25                  30
Trp Asp Val Thr Arg Ala Gly Arg Pro Gly Leu Gly Ala Gly Leu Arg
         35                  40                  45
Leu Pro Arg Leu Leu Ser Pro Pro Leu Arg Pro Arg Leu Leu Leu Leu
     50                  55                  60
Leu Leu Leu Leu Pro Pro Pro Leu Leu Leu Leu Leu Leu Pro Cys Glu
 65                  70                  75                  80
Ala Glu Ala Ala Ala Ala Ala Ala Ala Val Ser Gly Ser Ala Ala Ala
                 85                  90                  95
Glu Ala Lys Glu Cys Asp Arg Pro Cys Val Asn Gly Gly Arg Cys Asn
            100                 105                 110
Pro Gly Thr Gly Gln Cys Val Cys Pro Ala Gly Trp Val Gly Glu Gln
        115                 120                 125
Cys Gln His Cys Gly Gly Arg Phe Arg Leu Thr Gly Ser Ser Gly Phe
    130                 135                 140
Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr Lys Thr Lys Cys Thr Trp
145                 150                 155                 160
Leu Ile Glu Gly Gln Pro Asn Arg Ile Met Arg Leu Arg Phe Asn His
                165                 170                 175
Phe Ala Thr Glu Cys Ser Trp Asp His Leu Tyr Val Tyr Asp Gly Asp
            180                 185                 190
Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe Ser Gly Leu Ile Val Pro
        195                 200                 205
Glu Arg Asp Gly Asn Glu Thr Val Pro Glu Val Val Ala Thr Ser Gly
    210                 215                 220
Tyr Ala Leu Leu His Phe Phe Ser Asp Ala Ala Tyr Asn Leu Thr Gly
225                 230                 235                 240
Phe Asn Ile Thr Tyr Ser Phe Asp Met Cys Pro Asn Asn Cys Ser Gly
                245                 250                 255
Arg Gly Glu Cys Lys Ile Ser Asn Ser Ser Glu Thr Val Glu Cys Glu
            260                 265                 270
Cys Ser Glu Asn Trp Lys Gly Glu Ala Cys Asp Ile Pro His Cys Thr
        275                 280                 285
Asp Asn Cys Gly Phe Pro His Arg Gly Ile Cys Asn Ser Ser Asp Val
    290                 295                 300
Arg Gly Cys Ser Cys Phe Ser Asp Trp Gln Gly Pro Gly Cys Ser Val
305                 310                 315                 320
Pro Val Pro Ala Asn Gln Ser Phe Trp Thr Arg Glu Glu Tyr Ser Asn
                325                 330                 335
Leu Lys Leu Pro Arg Ala Ser His Lys Ala Val Val Asn Gly Asn Ile
            340                 345                 350
Met Trp Val Val Gly Gly Tyr Met Phe Asn His Ser Asp Tyr Asn Met
        355                 360                 365
Val Leu Ala Tyr Asp Leu Ala Ser Arg Glu Trp Leu Pro Leu Asn Arg
    370                 375                 380
Ser Val Asn Asn Val Val Val Arg Tyr Gly His Ser Leu Ala Leu Tyr
385                 390                 395                 400
Lys Asp Lys Ile Tyr Met Tyr Gly Gly Lys Ile Asp Pro Thr Gly Asn
                405                 410                 415
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Val Thr Asn Glu Leu Arg Val Phe His Ile His Asn Glu Ser Trp Val
420 425 430
Leu Leu Thr Pro Lys Ala Lys Glu Gln Tyr Ala Val Val Gly His Ser
435 440 445
Ala His Ile Val Thr Leu Lys Asn Gly Arg Val Val Met Leu Val Ile
450 455 460
Phe Gly His Cys Pro Leu Tyr Gly Tyr Ile Ser Asn Val Gln Glu Tyr
465 470 475 480
Asp Leu Asp Lys Asn Thr Trp Ser Ile Leu His Thr Gln Gly Ala Leu
485 490 495
Val Gln Gly Gly Tyr Gly His Ser Ser Val Tyr Asp His Arg Thr Arg
500 505 510
Ala Leu Tyr Val His Gly Gly Tyr Lys Ala Phe Ser Ala Asn Lys Tyr
515 520 525
Arg Leu Ala Asp Asp Leu Tyr Arg Tyr Asp Val Asp Thr Gln Met Trp
530 535 540
Thr Ile Leu Lys Asp Ser Arg Phe Phe Arg Tyr Leu His Thr Ala Val
545 550 555 560
Ile Val Ser Gly Thr Met Leu Val Phe Gly Gly Asn Thr His Asn Asp
565 570 575
Thr Ser Met Ser His Gly Ala Lys Cys Phe Ser Ser Asp Phe Met Ala
580 585 590
Tyr Asp Ile Ala Cys Asp Arg Trp Ser Val Leu Pro Arg Pro Asp Leu
595 600 605
His His Asp Val Asn Arg Phe Gly His Ser Ala Val Leu His Asn Ser
610 615 620
Thr Met Tyr Val Phe Gly Gly Phe Asn Ser Leu Leu Leu Ser Asp Ile
625 630 635 640
Leu Val Phe Thr Ser Glu Gln Cys Asp Ala His Arg Ser Glu Ala Ala
645 650 655
Cys Leu Ala Ala Gly Pro Gly Ile Arg Cys Val Trp Asn Thr Gly Ser
660 665 670
Ser Gln Cys Ile Ser Trp Ala Leu Ala Thr Asp Glu Gln Glu Glu Lys
675 680 685
Leu Lys Ser Glu Cys Phe Ser Lys Arg Thr Leu Asp His Asp Arg Cys
690 695 700
Asp Gln His Thr Asp Cys Tyr Ser Cys Thr Ala Asn Thr Asn Asp Cys
705 710 715 720
His Trp Cys Asn Asp His Cys Val Pro Arg Asn His Ser Cys Ser Glu
725 730 735
Gly Gln Ile Ser Ile Phe Arg Tyr Glu Asn Cys Pro Lys Asp Asn Pro
740 745 750
Met Tyr Tyr Cys Asn Lys Lys Thr Ser Cys Arg Ser Cys Ala Leu Asp
755 760 765
Gln Asn Cys Gln Trp Glu Pro Arg Asn Gln Glu Cys Ile Ala Leu Pro
770 775 780
Glu Asn Ile Cys Gly Ile Gly Trp His Leu Val Gly Asn Ser Cys Leu
785 790 795 800
Lys Ile Thr Thr Ala Lys Glu Asn Tyr Asp Asn Ala Lys Leu Phe Cys
805 810 815
Arg Asn His Asn Ala Leu Leu Ala Ser Leu Thr Thr Gln Lys Lys Val
820 825 830
Glu Phe Val Leu Lys Gln Leu Arg Ile Met Gln Ser Ser Gln Ser Met
835 840 845
Ser Lys Leu Thr Leu Thr Pro Trp Val Gly Leu Arg Lys Ile Asn Val
850 855 860
Ser Tyr Trp Cys Trp Glu Asp Met Ser Pro Phe Thr Asn Ser Leu Leu
865 870 875 880
Gln Trp Met Pro Ser Glu Pro Ser Asp Ala Gly Phe Cys Gly Ile Leu
885 890 895
Ser Glu Pro Ser Thr Arg Gly Leu Lys Ala Ala Thr Cys Ile Asn Pro
900 905 910
Leu Asn Gly Ser Val Cys Glu Arg Pro Ala Asn His Ser Ala Lys Gln
915 920 925
Cys Arg Thr Pro Cys Ala Leu Arg Thr Ala Cys Gly Asp Cys Thr Ser
930 935 940

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Gly Ser Ser Glu Cys Met Trp Cys Ser Asn Met Lys Gln Cys Val Asp
945                 950                 955                 960
Ser Asn Ala Tyr Val Ala Ser Phe Pro Phe Gly Gln Cys Met Glu Trp
                965                 970                 975
Tyr Thr Met Ser Thr Cys Pro Pro Glu Asn Cys Ser Gly Tyr Cys Thr
            980                 985                 990
Cys Ser His Cys Leu Glu Gln Pro Gly Cys Gly Trp Cys Thr Asp Pro
        995                 1000                1005
Ser Asn Thr Gly Lys Gly Lys Cys Ile Glu Gly Ser Tyr Lys Gly Pro
    1010                1015                1020
Val Lys Met Pro Ser Gln Ala Pro Thr Gly Asn Phe Tyr Pro Gln Pro
1025                1030                1035                1040
Leu Leu Asn Ser Ser Met Cys Leu Glu Asp Ser Arg Tyr Asn Trp Ser
                1045                1050                1055
Phe Ile His Cys Pro Ala Cys Gln Cys Asn Gly His Ser Lys Cys Ile
            1060                1065                1070
Asn Gln Ser Ile Cys Glu Lys Cys Glu Asn Leu Thr Thr Gly Lys His
        1075                1080                1085
Cys Glu Thr Cys Ile Ser Gly Phe Tyr Gly Asp Pro Thr Asn Gly Gly
    1090                1095                1100
Lys Cys Gln Pro Cys Lys Cys Asn Gly His Ala Ser Leu Cys Asn Thr
1105                1110                1115                1120
Asn Thr Gly Lys Cys Phe Cys Thr Thr Lys Gly Val Lys Gly Asp Glu
                1125                1130                1135
Cys Gln Leu Cys Glu Val Glu Asn Arg Tyr Gln Gly Asn Pro Leu Arg
            1140                1145                1150
Gly Thr Cys Tyr Tyr Thr Leu Leu Ile Asp Tyr Gln Phe Thr Phe Ser
        1155                1160                1165
Leu Ser Gln Glu Asp Asp Arg Tyr Tyr Thr Ala Ile Asn Phe Val Ala
    1170                1175                1180
Thr Pro Asp Glu Gln Asn Arg Asp Leu Asp Met Phe Ile Asn Ala Ser
1185                1190                1195                1200
Lys Asn Phe Asn Leu Asn Ile Thr Trp Ala Ala Ser Phe Ser Ala Gly
                1205                1210                1215
Thr Gln Ala Gly Glu Glu Met Pro Val Val Ser Lys Thr Asn Ile Lys
            1220                1225                1230
Glu Tyr Lys Asp Ser Phe Ser Asn Glu Lys Phe Asp Phe Arg Asn His
        1235                1240                1245
Pro Asn Ile Thr Phe Phe Val Tyr Val Ser Asn Phe Thr Trp Pro Ile
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Lys Ile Gln Val Gln Thr Glu Gln
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  1               5                  10                  15

gcg gcg acg gca gcg ctc gcg ggc agg agc ggc ggg ccg cac tgg gac      96
Ala Ala Thr Ala Ala Leu Ala Gly Arg Ser Gly Gly Pro His Trp Asp
             20                  25                  30

tgg gac gtg acc agg gct ggg agg ccg ggg ctg ggg gcc ggg ctg cgc     144
Trp Asp Val Thr Arg Ala Gly Arg Pro Gly Leu Gly Ala Gly Leu Arg
         35                  40                  45
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ctc ccg cgg ctg ctg tct cca ccg ctg cgg cca cgg ctg ctg ctg ctg      192
Leu Pro Arg Leu Leu Ser Pro Pro Leu Arg Pro Arg Leu Leu Leu Leu
     50                  55                      60

ctg ttg ttg ctc ccg ccg ccg ctg ttg ctg ctg ctg ctg ccc tgt gag      240
Leu Leu Leu Leu Pro Pro Pro Leu Leu Leu Leu Leu Leu Pro Cys Glu
 65                  70                  75                  80

gcc gag gcc gcg gcg gcg gcg gcg gcg gtg tcg ggc tca gcc gca gcc      288
Ala Glu Ala Ala Ala Ala Ala Ala Ala Val Ser Gly Ser Ala Ala Ala
                 85                  90                  95

gag gcc aag gaa tgt gac cgg ccc tgt gtc aac ggc ggt cgc tgc aac      336
Glu Ala Lys Glu Cys Asp Arg Pro Cys Val Asn Gly Gly Arg Cys Asn
            100                 105                 110

cct ggc acc ggc cag tgc gtc tgc ccc gcc ggc tgg gtg ggc gag caa      384
Pro Gly Thr Gly Gln Cys Val Cys Pro Ala Gly Trp Val Gly Glu Gln
        115                 120                 125

tgc cag cac tgc ggg ggc cgc ttc aga cta act gga tct tct ggg ttt      432
Cys Gln His Cys Gly Gly Arg Phe Arg Leu Thr Gly Ser Ser Gly Phe
    130                 135                 140

gtg aca gat gga cct gga aat tat aaa tac aaa acg aag tgc acg tgg      480
Val Thr Asp Gly Pro Gly Asn Tyr Lys Tyr Lys Thr Lys Cys Thr Trp
145                 150                 155                 160

ctc att gaa gga cag cca aat aga ata atg aga ctt cgt ttc aat cat      528
Leu Ile Glu Gly Gln Pro Asn Arg Ile Met Arg Leu Arg Phe Asn His
                165                 170                 175

ttt gct aca gag tgt agt tgg gac cat tta tat gtt tat gat ggg gac      576
Phe Ala Thr Glu Cys Ser Trp Asp His Leu Tyr Val Tyr Asp Gly Asp
            180                 185                 190

tca att tat gca ccg cta gtt gct gca ttt agt ggc ctc att gtt cct      624
Ser Ile Tyr Ala Pro Leu Val Ala Ala Phe Ser Gly Leu Ile Val Pro
        195                 200                 205

gag aga gat ggc aat gag act gtc cct gag gtt gtt gcc aca tca ggt      672
Glu Arg Asp Gly Asn Glu Thr Val Pro Glu Val Val Ala Thr Ser Gly
    210                 215                 220

tat gcc ttg ctg cat ttt ttt agt gat gct gct tat aat ttg act gga      720
Tyr Ala Leu Leu His Phe Phe Ser Asp Ala Ala Tyr Asn Leu Thr Gly
225                 230                 235                 240

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20948

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; C12N 15/63, 1/21, 15/00
US CL :536/23.5, 23.4; 435/320.1, 252.1, 69.7
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 23.4; 435/320.1, 252.1, 69.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, medline, embase, BIOSIS, caplus,
kEYWORDS: attractin, DPPT-L

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DUKE-COHAN et al. A Novel Form of Dipeptidylpeptidase IV Found in Human Serum. J. Biol. Chem. June 1995, Vol. 270, No. 23, pages 14107-14114, see entire document.	1-3, 6, 20-27
Y	DUKE-COHAN et al. Serum High Molecular Weight Dipeptidyl Peptidase IV (CD26) Is Similar To A Novel Antigen DPPT-L Released From Activated T Cells. J. Immunol. 1996, Vol. 156, pages 1714-1721, see entire document.	1-3, 6, 20-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 JANUARY 2000	07 FEB 2000

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Laurence Fa GERALD R. EWOLDT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT	International application No. PCT/US99/20948

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6, 20-27

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.
Group I, claims 1-3, 6, and 20-27, drawn to a DNA encoding a polypeptide.
Group II, claims 4-5 and 33-36, drawn to a polypeptide.
Group III, claim 7, drawn to a method of enhancing spreading of a macrophage or monocyte in vitro.
Group IV, claims 8-14, 32, and 37, drawn to a method of treating a mammal in need of an enhanced immune response.
Group V, claims 15-19, drawn to a method of inhibiting spreading of a macrophage or a monocyte in a mammal.
Group VI, claim 28, drawn to a method of identifying a compound that inhibits an immune response.
Group VII, claim 29, drawn to a method of identifying a compound that enhances an immune response.
Group VIII, claims 30-31, drawn to an antibody.

The inventions listed as Groups I, II, III, IV, V, VI, VII and VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking Groups I-VIII appears to be that they all relate to attractin nucleic acids and polypeptides and methods of using the polypeptide.

However Duke-Cohan et al.(J. Biol. Chem. 270(23): 14107-14114, 1995) and Duke-Cohan et al. (J. Immunol. 156: 1714-1721, 1996) teach the isolation and characterization of the DPPT-L (i.e. attractin) (see entire documents). Given the art known, and well known practice of isolating nucleic acids encoding proteins of interest to determine the structure-function characterization of such nucleic acids and their respective proteins, at the time the invention was made as well as the motivation and expectation of success in isolating cDNA encoding DPPT-L ,as set forth in Duke-Cohan et al. (1995; see Discussion), it would have been obvious to isolate a nucleic acid that encodes and/or hybridizes to the claimed attractin sequences of claim 1. Therefore, the special technical feature linking the inventions of Groups I-VIII does not constitute a special technical feature as it does not define a contribution over the prior art.

The special technical feature of Group I is a DNA encoding a nucleic acid, vectors, and cells expressing fusion proteins.

The special technical feature of Group II is a polypeptide encoding an attractin.

The special technical feature of Group III is a method of using the polypeptide of Group II to enhance spreading of macrophages in vitro.

The special technical feature of Group IV is a method of using the polypeptide of Group II to treat a mammal in need of an enhanced immune response.

The special technical feature of Group V is a method of using the polypeptide of Group II to inhibit spreading of a macrophage or monocyte in a mammal.

The special technical feature of Group VI is a method of using the polypeptide of Group II to identify a compound that inhibits an immune response.

The special technical feature of Group VII is a method of using the polypeptide of Group II to identify a compound that enhances an immune response.

The special technical feature of Group VIII is an antibody to the polypeptide of Group II.

Accordingly, Groups I-VIII are not so linked by the same special technical feature as to form a single inventive concept.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The nucleic acids of Group I, the polypeptides of Group II, and the antibody of Group VIII are different products with different biochemical structures and different functions.
The methods of Groups III-V require different starting materials, employ different steps, and have different endpoints.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as

follows:
Group IV is drawn to a method of treating different diseases, i.e., immunodeficiency diseases or cancer.
Group V is drawn to a method of inhibiting the spread of a macrophage or a monocyte in a mammal suffering from different medical conditions, i.e., an autoimmune disease or a transplant.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: patients with immunodeficiency diseases, cancer, autoimmune diseases, and transplant patients suffer from conditions with different etiologies, pathologies, and endpoints.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20948

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; C12N 15/63, 1/21, 15/00
US CL :536/23.5, 23.4; 435/320.1, 252.1, 69.7
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.5, 23.4; 435/320.1, 252.1, 69.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, medline, embase, BIOSIS, caplus,
kEYWORDS: attractin, DPPT-L

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DUKE-COHAN et al. A Novel Form of Dipeptidylpeptidase IV Found in Human Serum. J. Biol. Chem. June 1995, Vol. 270, No. 23, pages 14107-14114, see entire document.	1-3, 6, 20-27
Y	DUKE-COHAN et al. Serum High Molecular Weight Dipeptidyl Peptidase IV (CD26) Is Similar To A Novel Antigen DPPT-L Released From Activated T Cells. J. Immunol. 1996, Vol. 156, pages 1714-1721, see entire document.	1-3, 6, 20-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 JANUARY 2000	07 FEB 2000

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Laurence for GERALD R. EWOLDT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20948

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6, 20-27

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.
Group I, claims 1-3, 6, and 20-27, drawn to a DNA encoding a polypeptide.
Group II, claims 4-5 and 33-36, drawn to a polypeptide.
Group III, claim 7, drawn to a method of enhancing spreading of a macrophage or monocyte in vitro.
Group IV, claims 8-14, 32, and 37, drawn to a method of treating a mammal in need of an enhanced immune response.
Group V, claims 15-19, drawn to a method of inhibiting spreading of a macrophage or a monocyte in a mammal.
Group VI, claim 28, drawn to a method of identifying a compound that inhibits an immune response.
Group VII, claim 29, drawn to a method of identifying a compound that enhances an immune response.
Group VIII, claims 30-31, drawn to an antibody.

The inventions listed as Groups I, II, III, IV, V, VI, VII and VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking Groups I-VIII appears to be that they all relate to attractin nucleic acids and polypeptides and methods of using the polypeptide.

However Duke-Cohan et al.(J. Biol. Chem. 270(23): 14107-14114, 1995) and Duke-Cohan et al. (J. Immunol. 156: 1714-1721, 1996) teach the isolation and characterization of the DPPT-L (i.e. attractin) (see entire documents). Given the art known, and well known practice of isolating nucleic acids encoding proteins of interest to determine the structure-function characterization of such nucleic acids and their respective proteins, at the time the invention was made as well as the motivation and expectation of success in isolating cDNA encoding DPPT-L ,as set forth in Duke-Cohan et al. (1995; see Discussion), it would have been obvious to isolate a nucleic acid that encodes and/or hybridizes to the claimed attractin sequences of claim 1. Therefore, the special technical feature linking the inventions of Groups I-VIII does not constitute a special technical feature as it does not define a contribution over the prior art.

The special technical feature of Group I is a DNA encoding a nucleic acid, vectors, and cells expressing fusion proteins.

The special technical feature of Group II is a polypeptide encoding an attractin.

The special technical feature of Group III is a method of using the polypeptide of Group II to enhance spreading of macrophages in vitro.

The special technical feature of Group IV is a method of using the polypeptide of Group II to treat a mammal in need of an enhanced immune response.

The special technical feature of Group V is a method of using the polypeptide of Group II to inhibit spreading of a macrophage or monocyte in a mammal.

The special technical feature of Group VI is a method of using the polypeptide of Group II to identify a compound that inhibits an immune response.

The special technical feature of Group VII is a method of using the polypeptide of Group II to identify a compound that enhances an immune response.

The special technical feature of Group VIII is an antibody to the polypeptide of Group II.

Accordingly, Groups I-VIII are not so linked by the same special technical feature as to form a single inventive concept.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The nucleic acids of Group I, the polypeptides of Group II, and the antibody of Group VIII are different products with different biochemical structures and different functions.
The methods of Groups III-V require different starting materials, employ different steps, and have different endpoints.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as

follows:
Group IV is drawn to a method of treating different diseases, i.e., immunodeficiency diseases or cancer.
Group V is drawn to a method of inhibiting the spread of a macrophage or a monocyte in a mammal suffering from different medical conditions, i.e., an autoimmune disease or a transplant.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: patients with immunodeficiency diseases, cancer, autoimmune diseases, and transplant patients suffer from conditions with different etiologies, pathologies, and endpoints.